6TH EDITION

DIAGNOSTIC MEDICAL PARASITOLOGY

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Cover: Dog tapeworm (*Taenia pisiformis*), photograph taken using a light microscope, showing scolex with hooks. Spike Walker, Wellcome Images.

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Dedication

As with the first five editions, I dedicate this book to Marietta Voge, a truly rare individual who was widely recognized as one of the world's leading parasitologists. During her years as a diagnostic and research parasitologist at the University of California, Los Angeles, she touched the lives of many students and staff in a very special way. She was always more than willing to share her expertise with all who asked and volunteered this help over the years whenever contacted. She was always willing to donate a considerable amount of her personal time as a volunteer for various medical projects throughout the world.

She was a very special individual to work with, always interested in the person as well as the problem at hand. Her areas of teaching extended far beyond science. Whatever subject she was interested in received her total enthusiasm and dedication, and she had an exceptional ability to deal with detailed work. Her sense of fairness and professional integrity were remarkable; these ideals were shared with all who came in contact with her.

Her contributions to the field of diagnostic parasitology were numerous and included many classes, seminars, papers, and textbooks. The importance of working with Dr. Voge is hard to put into words. She was unique in her ability to allow a student to grow, both scientifically and personally. She could guide without constraints, teach without formal lectures, counsel without being judgmental, challenge without being unrealistic, tease without being cruel, and always be supportive regardless of the situation. She expected much from her students and employees and yet always gave considerably more than she received.

Scientific information gained from our association with her was invaluable; however, her impact on our lives was considerably more than scientific. She was always available for consultations and just to talk. She left all of us with a sense of having personally matured as a result of knowing and working with her over the years. She is missed by all of us, and yet her contributions in terms of teaching, consultations, volunteer work, professionalism, and friendship will remain with us forever.

I would also like to dedicate the sixth edition of this book to the bench technologists, those of you who provide critical diagnostic information on a daily basis and contribute such valuable input for excellent patient care.

Academic training provides key information in the field, but those who perform routine work at the bench often contribute much more than simple diagnostic identifications. Congratulations and thanks to all of you.

Finally, I also dedicate this book to John Lawrence. He was an extraordinary individual, and without his original encouragement and assistance, the first edition of the book would never have been written.

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Preface

During the past few years, the field of diagnostic medical parasitology has seen dramatic changes, including newly recognized parasites, emerging pathogens in new geographic areas, bioterrorism considerations and requirements, alternative techniques required by new regulatory requirements, reevaluation of diagnostic test options and ordering algorithms, continuing changes in the laboratory test menus, implementation of testing based on molecular techniques, reporting formats and report comments, coding and billing requirements, managed-care relevancy, increased need for consultation and educational initiatives for clients, and an overall increased awareness of parasitic infections from a worldwide perspective. We have seen organisms like the microsporidia change from the status of "unusual parasitic infection" to being widely recognized as among the most important infections in both immunocompetent and compromised patients. With confirmation of the fifth human malaria, *Plasmodium knowlesi*, this field has expanded dramatically. More sensitive diagnostic methods for organism detection in stool specimens are now commercially available for *Entamoeba histolytica*, *Entamoeba histolytica/E. dispar*, *Giardia lamblia*, Cryptosporidium spp., and *Trichomonas vaginalis*. Reagents are actively being developed for other organisms such as *Dientamoeba fragilis*, *Blastocystis* spp., and the microsporidia. We have seen *Cyclospora cayetanensis* coccidia become well recognized as the cause of diarrhea in immunocompetent and immunocompromised humans. We continue to see new disease presentations in compromised patients; a good example is granulomatous amebic encephalitis caused by *Acanthamoeba* spp., *Sappinia diploidea*, and *Balamuthia mandrillari*s. With the expansion of transplantation options, many parasites are potential threats to patients who are undergoing immunosuppression, and these must be considered within the context of this patient group. Transfusion transmission of potential parasitic pathogens continues to be problematic. Transfusion in general is becoming more widely recognized as a source of infection, and donors are also more likely to come from many parasite-endemic areas of the world. It is also important to recognize the many neglected parasitic infections seen within the United States; indeed, the world continues to shrink in terms of infectious diseases.

With expanding regulatory requirements related to the disposal of chemicals, laboratories are continuing to review the use of mercury compounds as specimen fixatives and learning to become familiar with organism morphology when using substitute compounds. Permanent staining of fecal smears confirms that none of the substitute fixatives provide results of the same quality found with the use of mercuric chloride-based fixatives. However, the key issue is whether the intestinal parasites can be identified using these alternative fixatives, not how "perfect" they look. Many fixative options are now available, including single-vial collection systems, some of which are coupled with their own stains. Requirements also mandate that any laboratory using formalin must have formalin vapor monitored as both an 8-hour time-weighted average and 15-minute readings. Most laboratories are now familiar with the regulations on protection of health care workers from blood and other body fluids and have implemented specific changes that are no longer optional. Although laboratories were already using many of the safety recommendations, these regulations delineate in detail what must be done and documented. Regulatory information based on new shipping requirements is also included.

On the basis of excellent suggestions and comments, I have made the following changes in this new edition: (i) the chapter on case histories has been expanded and contains a large number of parasite medical case histories (case history, study questions, correct answer and discussion, and illustrative material); (ii) some of the life cycles have been redrawn, and new life cycles have been added; (iii) algorithms have been expanded; (iv) new tables and figures have been added throughout the book; (v) additional drawings and photographs have been added; (vi) extensive color images have replaced the black and white images; (vii) extensive updated text information is included, all of which was taken from a comprehensive literature review of all aspects of diagnostic medical parasitology; (viii) additional examples of unusual parasitic infections are included; (ix) the chapter on arthropods has been expanded and includes additional photographs and drawings and expanded text; (x) the chapter on the immunology of parasitic infections has been enlarged, and updated information on both antigen and antibody detection methods continues to be included in this edition; (xi) the chapter on histological identification of parasites has been dramatically expanded with diagrams of various parasites and their visual presentations in tissue sections, with greatly enhanced legends for all images; (xii) diagnostic methods using newer immunoassay and "dipstick" technology are included; and (xiii) the chapter on quality control has been expanded to include information on instrumentation and equipment, safety regulations, quality control and quality systems information, continuous quality improvement, and managed-care considerations. The appendixes have been expanded to contain more information on artifacts; expanded lists and photographs of products and commercial suppliers; algorithms for ordering specific tests that complement the ova and parasite examination; flowcharts for processing stool specimens; quality control recording sheets for use in the laboratory; and general references and relevant web sites. One of the most important expanded areas of the sixth edition is found in Appendix 7, which contains information that has been published within months prior to the final printing of this edition. This "late-breaking" synopsis of very recent publications can assist the reader in having access to the latest information available. I encourage you to review this section as you read various chapters throughout the book. A more comprehensive discussion of molecular methods has also been added to the sixth edition and can be found in Appendix 8. Appendix 9 contains comprehensive information on the most frequently asked questions for all aspects of human parasitology, and Appendix 10 contains information related to CPT coding for testing options for diagnostic parasitology.

The approach to the sixth edition of the book has been revised to present the diagnostic methods first, then the didactic discussion of parasitic infections as the second component of the book. This change was made to ensure that the most recent and relevant material would be updated right before editing. My objective is to provide the user with clear, concise, well-organized, clinically relevant, cost-effective, and practical quality procedures for use in the clinical laboratory setting. To use and fully understand these methods for the parasites discussed, it is imperative that the user also understand information related to life cycle, morphology, clinical disease, pathogenesis, diagnosis, treatment, epidemiology, and prevention. My intent is to provide a comprehensive discussion of both aspects of the field of diagnostic medical parasitology: first, relevant diagnostic methods designed to detect and identify the organisms present, and second, a comprehensive discussion of the individual parasites. I believe that the book fulfills these objectives and provides readers, whether they are laboratorians, physicians, or other health care professionals, with not only comprehensive, but very practical information.

It is also important for readers to understand that there are many diagnostic test options available to the clinical laboratory; not every laboratory will approach the diagnosis of parasitic infections in the same way. The key to quality and clinically relevant diagnostic work is a thorough understanding of the pros and cons of each option and how various options may or may not be relevant for one's particular geographic area, laboratory size and range of expertise, client base, number and type of patients seen, personnel expertise and availability, equipment availability, educational initiatives, and communication options, just to name a few variables. However, it is also important to understand the regulations and technical recommendations that govern and guide this type of laboratory work; many of these guidelines are related to coding and reimbursement, proficiency testing, and overall clinical relevance.

The use of product names is not intended to endorse specific products or to exclude substitute products. Also, because of possible advances and changes in the therapy of parasitic infections, independent verification of drugs and drug dosages is always recommended. The diagnostic procedures are intended for laboratory use only by qualified and experienced individuals or by the personnel under their direct supervision. Every effort has been made to ensure accuracy; however, ASM Press and I encourage you to submit to us any suggestions, comments, and information on errors found.

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PART I

Diagnostic Procedures

1

Philosophy and Approach to Diagnostic Parasitology

With the expansion of world travel and increased access to more varied geographic areas and populations, medical and laboratory professionals will continue to see more "tropical" diseases and infections in nonendemic areas. This is due to the rapidity with which both people and organisms can be conveyed from one place to another. Travel has become available and more affordable for many people throughout the world, including those who are in some way compromised in terms of their overall health status. The increased transportation of infectious agents, as well as potential human carriers, has been clearly demonstrated, particularly via air travel. It has also been well documented that vectors carrying parasitic organisms can be transported via air travel in baggage and in the unpressurized parts of the plane itself; once released, these infected vectors can then transmit these parasites to humans, even in nonendemic areas.

With the continued increase in the number of patients whose immune systems are compromised through either underlying illness, chemotherapy, transplantation, AIDS, or age, we are much more likely to see increasing numbers of opportunistic infections, including those caused by parasites. Also, we continue to discover and document organisms once thought to be nonpathogenic that, when found in the compromised host, can cause serious disease. In considering the potential causes of illness in this patient population, the possibility of parasitic infections must always be considered as part of the differential diagnosis.

Diagnostic procedures in the field of medical parasitology require a great deal of judgmental and interpretative experience and are, with very few exceptions, classified by the Clinical Laboratory Improvement Act of 1988 (CLIA '88) as high complexity procedures. Very few procedures are automated, and organism identification relies primarily on morphologic characteristics that can be very difficult to differentiate. Although parasite morphology can be "learned" at the microscope, knowledge about the life cycle, epidemiology, infectivity, geographic range, clinical symptoms, range of illness, disease presentation depending on immune status, and recommended therapy is critical to the operation of any laboratory providing diagnostic services in medical parasitology.

The basic approach to diagnostic parasitology should be no different from that used in other areas of microbiology. There are guidelines published by the American Society for Microbiology (1–5), the American Society of Parasitologists (6), the American Society for Medical Technology (7), the College of American Pathologists (8), and the Clinical and Laboratory Standards Institute (formerly

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National Committee for Clinical Laboratory Standards) (9–16) that contain recommended procedures for this field. If these general guidelines and recommendations are not followed, there is some question as to the qualifications of the laboratory performing the diagnostic work. At the very least, the clinician should be informed about the limitations of the procedures that are being used. These guidelines are also accompanied by specific regulations for a number of laboratory issues and include CLIA '88 and requirements related to safety and protection of employees from blood or blood-borne pathogens (standard precautions) (17–20).

Because it is difficult for medical staff to maintain expertise in every available diagnostic procedure within microbiology, it is mandatory that close communication exist between the laboratory and clinicians. Frequent and complete communication, particularly concerning appropriate test orders and the clinical relevance of any diagnostic procedure within the context of total patient care and quality assurance, is very important. Therapeutic intervention often depends on results obtained from these procedures; therefore, the clinician must be aware of the limitations of each test method and the results obtained. This information becomes particularly important when one is discussing the patient's history and the recommended number and types of specimens to be submitted for examination.

During the past few years, there has been an increased awareness of the importance of having trained and qualified personnel perform these diagnostic procedures. There has been a concerted effort among many individuals and institutions in this country to upgrade the level of teaching and to bring to the medical community's attention the need for individuals who are familiar with diagnostic parasitology. With many laboratories decreasing staff size as a cost-containment measure, we are also seeing more "generalists" who are rotating throughout many sections of the laboratory, not just microbiology. Although necessary because of managed-care constraints and continued growth of capitated contracts, this approach contributes to the difficulties in maintaining well-trained staff in some of the specialty areas of microbiology. It becomes even more important to provide well-written laboratory protocols and to standardize test methods for consistency. There has also been increased awareness within the medical community of the need for additional training in the area of infectious diseases for the clinician and laboratory technician alike. This need has been reflected in the number of workshops, seminars, and publications that are available. The integration of information among all members of the health care team has certainly improved in terms of overall patient care.

The field of microbiology has taken on additional relevance and importance for a number of other reasons. Improved means of travel has made the world a smaller place. An individual's chances of exposure to parasites not endemic to his or her homeland and the possibility of acquiring or transmitting certain infections have been increasing. These facts emphasize the need to take a correct and complete history from a patient. It is important to be aware of the organisms commonly found within certain areas of the world and the makeup of the patient population being serviced at any particular health facility.

The most important step in the diagnosis of parasitic infections is the selection and submission of the appropriate clinical specimen within specified time lines and according to set protocols (21–23).

It is also important for the physician to know the efficacy of any diagnostic technique for parasite recovery and eventual diagnosis. Our approach to testing is undergoing continuous review, particularly within the current health care environment and cost-containment initiatives. The issue of patient care becomes particularly important when we begin to examine the number and types of compromised patients now being seen in all facilities. The increased publicity concerning immunocompromised patients has led to a greater awareness of parasitic infections in this patient population, regardless of the original cause of the immune deficiencies. Many of these patients with immune system defects are particularly at risk, whether because of previously acquired infections that have remained latent for many years or because of susceptibility to new infections. Many of these infections may present with unusual symptoms, and some are relatively new disease entities or those that are less commonly encountered (microsporidiosis, granulomatous amebic encephalitis, and infection with *Cyclospora cayetanensis*).

Often in other areas of microbiology, therapy is begun on the basis of patient history and symptoms. This approach is generally not recommended or used in cases of parasitic infection. Thus, the understanding of the characteristics of any parasitic infection (general geographic range, life cycle, clinical disease, diagnostic methods, therapy, epidemiology, and control) and the use of appropriate diagnostic procedures accompanied by a complete understanding of the limitations of each procedure become very important. Because of this approach to patient care, the general consensus among individuals within the field of diagnostic medical parasitology is that the use of certain incomplete procedures may result in incorrect information for the physician and may ultimately compromise patient care.

The main emphasis should be on the importance of understanding and recognizing potential parasitic infections, submitting the appropriate number and type of clinical specimens, knowing what procedures may provide confirmation of the diagnosis, and recognizing the implications and limitations of information provided to the physician. With the current emphasis on the development and use of molecular methods, understanding the benefits and limitations of these procedures will be critical to patient care outcomes. If there is an incomplete understanding of the requirements for high-quality diagnostic testing, incomplete information will be transmitted to the clinician. It is the responsibility of both the laboratory and the clinician to develop a greater awareness of the importance of these requirements.

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2

Collection, Preservation, and Shipment of Fecal Specimens

Safety

Fresh-specimen collection

- Collection of the specimen
- Number of specimens to be collected (standard recommendation)
- Number of specimens to be collected (pros and cons of various options)
- Collection times
- Specimen type, specimen stability, and need for preservation

Preservation of specimens

- Preservatives
  - Formalin
  - MIF
  - SAF
  - Schaudinn's fluid
  - Schaudinn's fluid containing PVA (mercury base)
  - Schaudinn's fluid containing PVA (copper base, zinc base)
  - Single-vial collection systems (other than SAF)
  - Universal Fixative (TOTAL-FIX)
- Use of fixatives
  - Quality control for stool fixatives
  - Procedure notes for use of preservatives
  - Procedure limitations for use of preservatives

Shipment of diagnostic specimens, biological products, etiologic agents, or infectious substances

- Documentation

This chapter discusses various collection methods that are available for specimens suspected of containing parasites or parasitic elements. When a laboratory selects its collection methods, the decision should be based on a thorough understanding of the value and limitations of each. One of the most important aspects of specimen collection is that the final laboratory results based on parasite recovery and identification will depend on the initial fixation of the organisms (1–3). Unless the appropriate specimens are properly collected and processed, these infections may not be detected (1). Considering the current era of cost containment and review of clinical relevance of laboratory information generated, specimen rejection criteria have become more important within the context of all diagnostic microbiology procedures. Diagnostic laboratory results based on improperly collected specimens may require excessive expenditures of time and supplies and may also mislead the physician. As a part of any overall total quality management or continuous quality improvement program for the laboratory, the generation of test results must begin with stringent criteria for specimen acceptance or rejection.

Clinically relevant diagnostic parasitology testing also depends on receiving appropriate test orders from the physician. Depending on the patient's clinical condition and travel history, very specific diagnostic tests may be recommended. It is extremely important that physician clients are aware of the test order options available within the laboratory test menu and the pros and cons of each test when considered within the context of the patient's history and symptoms. Without the proper test orders, diagnostic test results may be misleading or actually incorrect. Appropriate and complete communication regarding test orders between the laboratory and physicians is mandatory for high-quality patient care.

Safety

All fresh specimens should be handled carefully, since each specimen represents a potential source of infectious material (bacteria, viruses, fungi, and parasites). Safety precautions should include awareness of the following: proper labeling of fixatives; specific areas designated for specimen handling (biological safety cabinets may be necessary under certain circumstances); proper containers for centrifugation; acceptable discard policies; appropriate policies for no eating,

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drinking, or smoking, etc., within the working areas; and, if applicable, correct techniques for organism culture and/or animal inoculation.

Since diagnostic parasitology work is most often performed within the microbiology division of a clinical laboratory, all general guidelines for safety would also apply. Any special precautions which apply to a particular technique are discussed in the following chapters. In general, standard precautions as outlined by the Occupational Safety and Health Act must be followed when applicable, particularly when one is handling blood and other body fluids (4).

Fresh-Specimen Collection

Procedures for the recovery of intestinal parasites should always be performed before barium is used for radiological examination. Stool specimens containing barium are unacceptable for examination, and intestinal protozoa may be undetectable for 5 to 10 days after barium is given to the patient. There are also certain substances and medications that interfere with the detection of intestinal protozoa: mineral oil, bismuth, antibiotics, antimalarial agents, and nonabsorbable antidiarrheal preparations. After administration of any of these compounds, parasitic organisms may not be recovered for a week to several weeks. The two most commonly used substances are barium and antibiotics, such as tetracycline, which modify the gastrointestinal tract flora. Specimen collection should be delayed for 5 to 10 days or at least 2 weeks after barium or antibiotics, respectively, are administered (1, 2, 5–24). The use of antibacterial therapy that affects the normal gastrointestinal tract flora will diminish the numbers of protozoa, since they feed on intestinal bacteria.

Collection of the Specimen

Fecal specimens should be collected in clean, wide-mouth containers; often a 0.5-pint (ca. 0.24-liter) waxed cardboard or plastic container with a tight-fitting lid is selected for this purpose. The fit of the lid is particularly important, both from the standpoint of accidental spillage and in order to maintain moisture within the specimen. The specimens should not be contaminated with water or urine, because water may contain free-living organisms that can be mistaken for human parasites and urine may destroy motile organisms. For safety reasons, stool specimen containers should be placed in plastic bags when transported to the laboratory for testing. Fresh specimens can also be submitted in collection vials (Fig. 2.1). All fresh specimens should be carefully handled, since they are potential sources of infectious organisms, including bacteria, viruses, and parasites. Every specimen should be identified with the following minimal information: patient's name and identification number, physician's name, and the date and time the specimen was collected (if the laboratory is computerized, the date and time may reflect arrival in the laboratory, not the actual collection time). The specimen must also be accompanied by a request form indicating which laboratory procedures are to be performed. It is also be very helpful to have information concerning the presumptive diagnosis or relevant travel history; however, this information is rarely available, and under certain circumstances, the physician will have to be contacted for additional patient history (Example: Fever of unknown origin [FUO]—possible malaria).

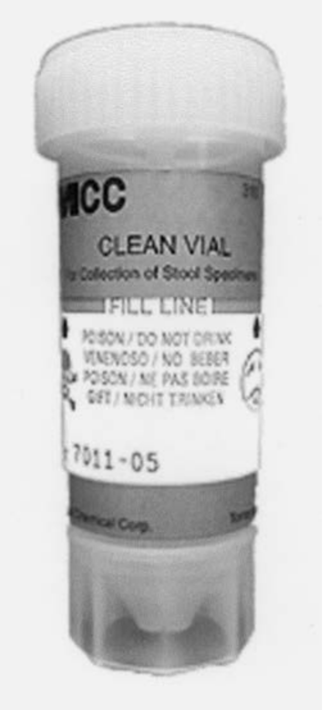


Figure 2.1 Stool collection vial; "clean vial" contains no fixatives. doi:10.1128/9781555819002.ch2.f2.1

Number of Specimens To Be Collected (Standard Recommendation)

It is recommended that a normal examination for stool parasites before therapy include three specimens, consisting of two specimens collected from normal movements and one collected after the use of a cathartic such as magnesium sulfate or Fleet's Phospho-Soda. A cathartic with an oil base should not be used, and a stool softener (taken either orally or as a suppository) is usually inadequate for obtaining a purged specimen. The purpose of the laxative is to stimulate some "flushing" action within the gastrointestinal tract, possibly allowing one to obtain more organisms

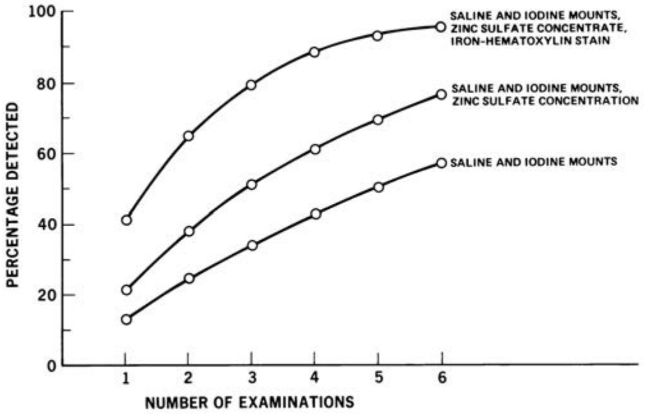


Figure 2.2 Increased detection of *Entamoeba histolytica* by using various diagnostic techniques and serial stool specimens. (Adapted by Markell EK, Voge M, John DT, 1992, *Medical Parasitology*, 7th ed., The W. B. Saunders Co., Philadelphia, PA, from references 17 and 31, with permission.) doi:10.1128/9781555819002.ch2.f2.2

for recovery and identification. Obviously, if the patient already has diarrhea or dysentery, the use of any laxatives would be contraindicated. Since the majority of patients are symptomatic prior to submission of stool specimens for examination, the need for a laxative is relatively uncommon.

When a patient is suspected of having intestinal amebiasis, six specimens may be recommended. The examination of six specimens ensures detection of approximately 90% of amebic infections (5) (Fig. 2.2). However, because of cost-containment measures, the examination of six specimens is rarely requested.

Three specimens are also recommended for posttherapy examinations, and they should be collected as outlined above. However, a patient who has received treatment for a protozoan infection should be checked 3 to 4 weeks after therapy, and those treated for *Taenia* infections should be checked 5 to 6 weeks after therapy. In some cases, the physician will assume a cure for tapeworm infection unless proglottids reappear in the stool; therefore, no posttherapy specimens are submitted for examination.

Number of Specimens To Be Collected (Pros and Cons of Various Options)

During the past few years, a number of issues have surfaced regarding the collection, processing, and testing of stool specimens for diagnostic parasitology. Many of the new suggestions and options have arisen as a result of continued cost-containment measures, limited reimbursement, and the elimination of mercury-based compounds for stool preservatives. The number of nonmercury preservative choices, collection systems, concentration devices, and immunoassays has increased dramatically. Many laboratories continue to review the options, and some may be having difficulties in selecting the proper approach (7–22).

It is important to realize that there are many acceptable options and that many laboratories will select different approaches. These differences should not be categorized as “right or wrong” or “acceptable or unacceptable”—they are merely different! To assume that there is only one correct approach for the examination of stool specimens is neither appropriate nor realistic. There are many parameters to consider before selecting the approach for your own laboratory. In no particular order, some of the considerations include client base, physician ordering patterns, number of specimens received per month, cost, presence or absence of appropriate equipment, current and possible methodologies (including immunoassays such as enzyme immunoassay [EIA], fluorescent-antibody assay [FA], and rapid membrane flow cartridge devices [rapids]), availability of expert microscopists, collection options, selection of preservative-stain combinations, reimbursement issues, client education, area of the world where laboratory is located, and emphasis on the most common infections (helminth or protozoa or both).

When considering available options and laboratory test menus, it is important to make sure that the pros and cons of the approaches selected are thoroughly understood and that diagnostic tests, potential results, and reporting formats are carefully explained to all clients. As an example, if the results of a stool examination are based on a concentration sediment examination only, this information must be conveyed to the physician. Many of the intestinal protozoa are missed by this diagnostic test approach, and it is important for the physician to recognize the limitations of such testing. Most physicians receive very little, if any, exposure to medical parasitology in medical school, and many newer physicians trained as generalists or family practitioners also have limited parasitology training or experience.

In most cases, it is probably realistic to assume that patients are symptomatic if they are submitting stool specimens for diagnostic parasitology testing. In an excellent article by Hiatt et al., the premise tested was that a single stool sample from a symptomatic patient would be sufficient to diagnose infections with intestinal protozoa (23). However, with additional stool examinations for symptomatic patients, the yield of intestinal protozoa increased dramatically (*Entamoeba histolytica*, 22.7% increase; *Giardia lamblia*, 11.3% increase; and *Dientamoeba fragilis*, 31.1% increase). This publication again demonstrates the problems with performing only a single stool examination (using the ova and parasite examination). If the patient becomes asymptomatic after the first stool examination, it may be acceptable to discontinue the series of stool examinations (this should be a clinician decision).

Available options are compared to the "gold standard," which includes a series of three stool examinations (direct, concentrate, and permanent stained smear for a fresh specimen; concentrate and permanent stained smear for a preserved specimen). The single-specimen pros and cons are discussed in the previous paragraph (symptomatic versus asymptomatic patients). A suggestion has been made to pool three specimens and to perform a single concentration and a single permanent stained smear. Depending on the number of positives and negatives, this may be a viable option, but some organisms may be missed. Another testing option for three pooled specimens might be to perform a single concentration and three separate permanent stained smears. This approach would probably increase the yield of intestinal protozoa over the previous option. Another suggestion involves placing a sample of each of three stools into a single vial of preservative. This collection approach would require only a single vial, but it is very likely that the vial would be overfilled and that mixing and the ratio of fixative to stool would be inaccurate.

One could also test patient specimens by using immunoassay reagents or kits for *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*), the *Entamoeba histolytica/E. dispar* group, *Entamoeba histolytica*, or *Cryptosporidium* spp. However, patient history information is rarely received with the clinical specimen; fecal testing based on patient risk group or situation is impossible without sufficient information. Testing of patient specimens by immunoassay procedures should be performed on request; however, client education is critical for successful implementation of this approach. Collection and testing options and the pros and cons of each are listed in Tables 2.1 and 2.2.

The Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) document *Procedures for the Recovery and Identification of Parasites from the Intestinal Tract* (Approved Guideline M28-2A) was updated in 2005. Various stool collection, processing, and testing options are also included in that publication (1, 25).

In summary, laboratories performing diagnostic parasitology testing must decide on the appropriate test methods that are relevant for their own operations based on a number of variables mentioned above. It is unrealistic to assume or state that one approach is applicable for every laboratory; however, it is important to thoroughly understand the options within your test menu and to convey this information to your clients once your approach has been selected for implementation. Prior discussion with clients, written educational memos, meetings, and examples of revised report formats are highly recommended prior to implementation.

Collection Times

A series of three specimens as indicated above should be submitted on separate days; if possible, the specimens should be submitted every other day; otherwise, the series of three specimens should be submitted within no more than 10 days. If a series of six specimens is requested, the specimens should also be collected on separate days or within no more than 14 days. Many organisms, particularly the intestinal protozoa, do not appear in the stool in consistent numbers on a daily basis, and the series of three specimens is considered a minimum for an adequate examination. It is inappropriate for multiple specimens to be submitted from the same patient on the same day. One possible exception would be stool collections from a patient who has severe, watery diarrhea such that any organisms present might be missed because of the tremendous dilution factor related to fluid loss. Even under these circumstances, acceptance of more than one specimen per patient per day should not be routine but should be done only after consultation with the physician. It is also not recommended for the three specimens to be submitted one each day for three consecutive days; however, use of this collection time frame would not be sufficient cause to reject the specimens. Adequate spacing between specimens helps to provide parasite recovery within the recommended time frames.

Although the recommended number of stool specimens is three, laboratories have been more willing to accept two specimens, primarily because of cost savings and the assumption that if the patient is symptomatic, confirmation of any organisms present is just as likely to be possible from two specimens as from three specimens. However, it is important that clients understand the pros and cons of two compared with three stools. Both collection approaches are being used by diagnostic laboratories.

Specimen Type, Specimen Stability, and Need for Preservation

Fresh specimens are mandatory for the recovery of motile trophozoites (amebae, flagellates, or ciliates). The protozoan trophozoite stage is normally found in cases of diarrhea; the gastrointestinal tract contents are moving through the system too rapidly for cyst formation to occur. Once the stool specimen is passed from the body, trophozoites do not encyst but may disintegrate if not examined or preserved within a short time after passage. The time limit recommendations listed below are most relevant for the intestinal protozoa; most helminth eggs

TABLE 2.1 Fecal specimens for parasites: options for collection and processing[a]

Option	Pros	Cons
Rejection of stools from inpatients who have been in the hospital for >3 days[a]	Data suggest that patients who begin to have diarrhea after they have been inpatients for a few days are symptomatic not from parasitic infections but generally from other causes.	There is always the chance that the problem is related to a health care-associated (nosocomial) parasitic infection (rare), but *Cryptosporidium* and microsporidia may be possible considerations.
Examination of a single stool specimen (O&P examination); data suggest that 40% of organisms present will be found with only a single stool exam; two O&P exams (concentration, permanent stained smear) are acceptable but are not always as good as three specimens (this may be a relatively cost-effective approach); any patient remaining symptomatic would require additional testing.	Some think that most intestinal parasitic infections can be diagnosed from examination of a single stool; if the patient becomes asymptomatic after collection of the first stool, subsequent specimens may not be necessary.	Diagnosis from a single stool examination depends on experience of the microscopist, proper collection, and the parasite load in the specimen; in a series of three stool specimens, it is often the case that not all three specimens are positive and/or may be positive for different organisms.
Examination of a second stool specimen only after the first is negative and the patient is still symptomatic	With additional examinations, the yield of protozoa increases (*Entamoeba histolytica*, 22.7%; *Giardia lamblia*, 11.3%; and *Dientamoeba fragilis*, 31.1%) (23).	Assumes the second (or third) stool specimen is collected within the recommended 10-day time frame for a series of stools (protozoa are shed periodically); may be inconvenient for patient.
Examination of a single stool and an immunoassay (EIA, FA, and lateral or vertical flow cartridge). This approach is a mix. One immunoassay may be acceptable; however, immunoassay testing of two separate specimens is required to confirm the presence of *Giardia* antigen. One O&P exam is not the best approach (review last option below).	If the examinations are negative and the patient's symptoms subside, probably no further testing is required.	Patients may exhibit symptoms (off and on), so it may be difficult to rule out parasitic infections with only a single stool and one fecal immunoassay. If the patient remains symptomatic, then even if two *Giardia* immunoassays are negative, other protozoa may be missed (the *Entamoeba histolytica/E. dispar* group, *Entamoeba histolytica*, *Dientamoeba fragilis*, *Blastocystis* spp., *Cryptosporidium* spp., the microsporidia). Normally, there are specific situations where fecal immunoassays *or* O&P exams should be ordered. It is not recommended to automatically perform both the O&P and the fecal immunoassay as a stool exam for parasites. *Depending on the patient's history and clinical symptoms, either the O&P exam* ***or*** *a fecal immunoassay may be recommended*, ***but generally not both.***
The laboratory will pool three specimens for examination; perform *one* concentration and examine *one* permanent stain.	Three specimens are collected by the patient (three separate collection vials) over 7–10 days; pooling by the laboratory may save time and expense.	Organisms present in low numbers may be missed due to the dilution factor once the specimens are pooled.
The laboratory will pool three specimens for examination; perform *one* concentration and examine *three* permanent stained smears.	Three specimens are collected by the patient (three separate collection vials) over 7–10 days; pooling by the laboratory for the concentration would probably be sufficient for the identification of helminth eggs. Examination of the three separate permanent stained smears (one from each vial) would maximize recovery of intestinal protozoa in areas of the country where these organisms are most common.	Might miss light helminth infection (eggs and larvae) due to the pooling of the three specimens for the concentration; however, with a permanent stain performed on each of the three specimens, this approach would probably be the next best option in lieu of the standard approach (concentration and permanent stained smear performed on every stool). Coding and billing would have to match the work performed; this may present some problems where work performed does not match existing codes.
The patient collects three stools but puts a sample of stool from all three specimens into a single vial (patient is given a single vial only).	Pooling of the specimens would require only a single vial.	This would complicate patient collection and very likely result in poorly preserved specimens, especially regarding the recommended ratio of stool to preservative and the lack of proper mixing of specimen and fixative.

(continued)

TABLE 2.1 *(continued)*

Option	Pros	Cons
Perform immunoassays on selected patients[b] by FA, EIA, or rapid cartridge methods for *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*), *Cryptosporidium* spp., and/or *Entamoeba histolytica*/*E. dispar* or *Entamoeba histolytica*.	Would be more cost-effective than performing immunoassay procedures on all specimens; however, the information needed to group patients is often not received with specimens. *This approach assumes that the physicians have guidance in terms of correct ordering options* (*see* Table 2.2).	Laboratories rarely receive information that would allow them to place a patient in a particular risk group, such as children <5 yr, children from day care centers (may or may not be symptomatic), patients with immunodeficiencies, and patients from outbreaks; performance of immunoassay procedures on every stool is not cost-effective, and the positive rate will be low unless an outbreak situation is involved.
Perform immunoassays and O&P examinations on request[c]: *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*), *Cryptosporidium* spp. and/or *Entamoeba histolytica*/*E. dispar* group, or *Entamoeba histolytica*. A number of variables will determine the approach to immunoassay testing and the O&P examination (geography, parasites recovered, positivity rate, and physician requests). Immunoassays and/or O&P examinations should be separately ordered, reported, and billed.	This approach will limit the number of stools on which immunoassay procedures for parasites are performed. Immunoassay results do not have to be confirmed by any other testing (such as O&P examinations or modified acid-fast stains). If specific kit performance problems have been identified, individual laboratories may prefer to do additional testing. *However, the fecal immunoassays are more sensitive than the O&P examination and special stains (modified acid-fast stains).* Also, this may be considered duplicate testing and may not be approved for reimbursement unless specifically ordered by the physician.	This approach will require education of the physician clients regarding appropriate times and patients for whom fecal immunoassays should be ordered. Educational initiatives must also include information on the test report indicating the pathogenic parasites that will *not* be detected using these methods. It is critical to make sure clients know that if patients have become asymptomatic, further testing may not be required. *However*, if the patient remains symptomatic, then further testing (O&P exams) is required. Remember, a single O&P may not reveal all organisms present. Present plan to physicians for approval: immunoassays or O&P examinations, procedure discussion, report formats, clinical relevance, and limitations on each approach.

[a] O&P, ova and parasite; EIA, enzyme immunoassay; FA, fluorescent-antibody immunoassay. See references 7, 23, and 35–37.

[b] See in particular references 7 and 23.

[c] See Table 2.2 on test ordering. Regarding ordering recommendations for routine O&P examinations or fecal immunoassays, it is difficult to know when you may be in an early outbreak situation where testing of all specimens for *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*), *Cryptosporidium* spp., or both may be relevant. Extensive efforts are under way to encourage communication among laboratories, water companies, pharmacies, and public health officials regarding the identification of potential or actual outbreaks. If it appears that an outbreak is in the early stages, then performing the immunoassays on request can be changed to testing all stools.

and larvae, coccidian oocysts, and microsporidian spores survive for extended periods. However, no one can predict which organisms will be present in the stool specimens; therefore, it is important to use the most conservative time frames for parasite recovery.

Liquid specimens should be examined within 30 min of passage, not 30 min from the time they reach the laboratory. If this general time recommendation of 30 min is not possible, the specimen should be placed in one of the available fixatives. Soft (semiformed) specimens may contain a mixture of protozoan trophozoites and cysts and should be examined within 1 h of passage; again, if this time frame is not possible, preservatives should be used. Immediate examination of formed specimens is not as critical; in fact, if the specimen is examined at any time within 24 h after passage, the protozoan cysts should still be intact (5, 16).

In review, remember that trophozoites only are usually found in liquid specimens, both protozoan trophozoites and cysts can be recovered in soft specimens, and generally cysts only are recovered in formed specimens. The time limits mentioned above are merely guidelines; however, if fresh specimens remain unpreserved for longer times before examination, many if not all organisms may disintegrate or become distorted. Fecal specimens should never be incubated or frozen prior to examination using routine microscopy. When the acceptance criteria for specimen collection are not met, the laboratory should reject the specimen and request additional specimens.

Because there is often a time lag from the time of specimen passage until receipt in the laboratory, many clinicians, clinics, and inpatient wards use a specimen collection system that includes stool preservatives (Table 2.3). A number of commercial systems are available with many preservative choices; the use of such systems has become routine for many institutions, and some request a custom collection kit that may contain several types of preservatives for stool specimens, depending on the tests normally ordered by the clinicians that they service. Specific information concerning collection kit components is provided in Appendix 1.

TABLE 2.2 Approaches to stool parasitology: test ordering[a]

Patient and/or situation	Test ordered[b]	Follow-up test ordered
Patient with diarrhea and AIDS (or other cause of immune deficiency) Potential waterborne outbreak (municipal water supply)	*Cryptosporidium* or *Giardia/Cryptosporidium* immunoassay	If immunoassays are negative and symptoms continue, special tests for microsporidia (modified trichrome stain) and other coccidia (modified acid-fast stain) and O&P exam should be performed.
Patient with diarrhea (nursery school, day care center, camper, backpacker) Patient with diarrhea and potential waterborne outbreak (resort setting) Patients with diarrhea who live within areas of the United States where *Giardia* is the most common organism seen	*Giardia* or *Giardia/Cryptosporidium* immunoassay	If immunoassays are negative and symptoms continue, special tests for microsporidia and other coccidia (see above) and O&P exam should be performed.
Patient with diarrhea and relevant travel history Patient with diarrhea who is a past or present resident of a developing country Patient in area of United States where parasites other than *Giardia* are found (large metropolitan centers such as New York, NY; Los Angeles, CA; Washington, DC; and Miami, FL)	O&P exam, *Entamoeba histolytica/E. dispar* immunoassay, confirmation for *E. histolytica*; various tests for *Strongyloides* may also be relevant (even in the absence of eosinophilia), particularly if there is any history of pneumonia (migrating larvae in lungs) or sepsis or meningitis (fecal bacteria carried by migrating larvae); agar plate culture is the most sensitive diagnostic approach for *Strongyloides*.	The O&P exam is designed to detect and identify a broad range of parasites (amebae, flagellates, ciliates, *Cytoisospora belli*, and helminths). If exams are negative and symptoms continue, special tests for coccidia (fecal immunoassays, modified acid-fast stains, or autofluorescence) and microsporidia (modified trichrome stains or calcofluor white stains) should be performed; fluorescent stains are also options.
Patient with unexplained eosinophilia	Although the O&P exam is recommended, agar plate culture for *Strongyloides stercoralis* (more sensitive than the O&P exam) is also recommended, particularly if there is any history of pneumonia (migrating larvae in lungs) or sepsis or meningitis (fecal bacteria carried by migrating larvae).	If tests are negative and symptoms continue, additional O&P exams and special tests for microsporidia and other coccidia should be performed.
Patient with diarrhea (suspected food-borne outbreak)	Test for *Cyclospora cayetanensis* (modified acid-fast stain, autofluorescence)	If tests are negative and symptoms continue, special procedures for microsporidia and other coccidia and O&P exam should be performed.

[a] O&P exam, ova and parasite examination.

[b] Depending on the particular immunoassay kit used, various single or multiple organisms may be included. Selection of a particular kit depends on many variables: clinical relevance, cost, ease of performance, training, personnel availability, number of test orders, training of physician clients, sensitivity, specificity, equipment, time to result, etc. Very few laboratories will handle this type of testing exactly the same. Many options are clinically relevant and acceptable for good patient care. It is critical that the laboratory report indicate specifically which organisms could be identified using the kit; a negative report should list the organisms relevant to that particular kit. *It is important to remember that sensitivity and specificity data for all of these fecal immunoassay kits (fluorescent-antibody immunoassay, enzyme immunoassay, and cartridge formats) are comparable.*

KEY POINTS—STOOL SPECIMEN COLLECTION

1. Occupational Safety and Health Act regulations (including standard precautions) should be used for handling all specimens.
2. Interfering substances (oil-based laxatives, barium, antibiotics) should be avoided when stool specimens are collected.
3. Contamination with urine or water should be avoided.
4. Recommendation for collection: three specimens collected, one every other day or within a 10-day time frame.
   Note: There are some exceptions; see Table 2.1.
5. Liquid stool: examine or preserve within 30 min of passage (trophozoites). Soft stool: examine or preserve within 1 h of passage (trophozoites and cysts). Formed stool: examine or preserve within 24 h of passage. Note that *Dientamoeba fragilis* trophozoites can be found in formed stool specimens as well as liquid specimens.

Preservation of Specimens

Preservatives

There are a number of reasons why a lag time may occur from the time of specimen passage until examination in the laboratory (e.g., the workload in the laboratory or

TABLE 2.3 Stool collection: pros and cons of fresh and preserved specimens[a]

Specimen	Pros	Cons
Fresh stool	1. No requirements for stool fixatives 2. Ability to see motile trophozoites 3. Lower cost (no fixative vials) 4. Can perform direct wet examination, concentration, permanent stained smear, fecal immunoassays, and special stains for coccidia and microsporidia 5. Acceptable for good organism morphology *only* if time from stool passage to laboratory is within the time limits indicated below: in symptomatic patients, the trophozoite form of the intestinal protozoa is present and will not encyst when outside of the body but will disintegrate. Time limits: liquid or watery stool, 30 min; semiformed stool, 1 h; formed stool, 24 h	1. May have excessive lag time between stool fixation or processing; trophozoites may disintegrate, thus giving a false-negative result. 2. Ova and parasite examination (direct wet examination, concentration, and permanent stained smear) may be negative due to lack of organism preservation and morphology integrity.
Preserved stool	1. Organism morphology is preserved when lag time between stool passage and fixation is short (this can be done by allowing patients to collect and fix stool specimens at home); once the specimen is mixed with the preservative, the delivery time to the laboratory is not critical. 2. Can perform concentration, permanent stain, fecal immunoassays, and special stains for coccidia and microsporidia. 3. Fixatives now available that contain no mercury, no formalin, and no PVA (Universal Fixatives)	1. Cost of collection vials may represent a cost increase; however, overall expense may be lower because of much more accurate result (patient outcome). 2. Disposal of vials may be a problem if the laboratory is using preservatives containing mercuric chloride.

[a] Most products used for specimen collection are available from any major medical supply house. PVA, polyvinyl alcohol; O&P, ova and parasite examination. Adapted from reference 2.

the transit distance or time for the specimen to reach the facility). To preserve protozoan morphology and to prevent the continued development of some helminth eggs and larvae, the stool specimens can be placed in preservative immediately after passage (by the patient using a collection kit) or once the specimen is received by the laboratory. There are several fixatives available; the more common ones, including formalin, Merthiolate (thimerosal)-iodine-formalin (MIF), sodium acetate-acetic acid-formalin (SAF), Schaudinn's fluid (with or without polyvinyl alcohol [PVA]), the single-vial systems, and the Universal Fixative, are discussed in detail (Table 2.4). Regardless of the fixative selected, adequate mixing of the specimen and preservative is mandatory. A flow diagram for preservation and processing is shown in Fig. 2.3.

Note When selecting an appropriate fixative, keep in mind that a permanent stained smear is mandatory for a complete examination for parasites (1, 2, 26). It is also important to remember that disposal regulations for compounds containing mercury are becoming more restrictive; each laboratory will have to check applicable state and federal regulations to help determine fixative options.

Note It is important to remember that when using fecal immunoassays for the *Entamoeba histolytica/E. dispar* group or *Entamoeba histolytica*, fresh or frozen stool is required (in some cases Cary-Blair may be acceptable, but check the package insert prior to use). To date, stool submitted in routine fecal fixatives is not acceptable for these particular fecal immunoassays.

Formalin

Formalin has been used for many years as an all-purpose fixative that is appropriate for helminth eggs and larvae and for protozoan cysts, oocysts, and spores. Two concentrations are commonly used: 5%, which is recommended for preservation of protozoan cysts, and 10%, which is recommended for helminth eggs and larvae. Although 5% is often recommended for all-purpose use, most commercial manufacturers provide 10%, which is more likely to kill all helminth eggs (Fig. 2.4). To help maintain organism morphology, the formalin can be buffered with sodium phosphate buffers, i.e., neutral formalin. Selection of specific formalin formulations is at the user's discretion. *Aqueous formalin will permit the examination of the specimen as a wet mount only, a technique much less accurate than a permanent stained smear for the identification of intestinal protozoa. However, the fecal immunoassays for* Giardia lamblia (G. duodenalis, G. intestinalis) *and* Cryptosporidium *spp. can be performed from the aqueous formalin*

TABLE 2.4 Preservatives used in diagnostic parasitology (stool specimens)[a]

Preservative	Concn	Permanent stained smear	Immunoassays[b] (*Giardia lamblia* [*G. duodenalis*, *G. intestinalis*], *Cryptosporidium* spp.)	Comments
5% or 10% formalin	Yes	No	Yes	EIA, FA, cartridge
5% or 10% buffered formalin	Yes	No	Yes	EIA, FA, cartridge
MIF	Yes	Polychrome IV stain	ND	No published data
SAF	Yes	Iron hematoxylin or trichrome (modified for SAF)	Yes	EIA, FA, cartridge
Schaudinn's fixative[c] (mercury base) with or without PVA	Yes, but rarely used	Trichrome or iron hematoxylin	No	PVA interferes with immunoassays
Modified Schaudinn's[d] (copper base) with PVA	Yes, but rarely used	Trichrome or iron hematoxylin	No	PVA interferes with immunoassays
Modified Schaudinn's[e] (zinc base) with PVA	Yes	Trichrome or iron hematoxylin	No	PVA interferes with immunoassays
Single-vial systems[f] (with or without PVA)	Yes	Trichrome or iron hematoxylin	Some, but not all (no if contains PVA)	Check with the manufacturer
Universal Fixative[g] (single-vial system)	Yes	Trichrome or iron hematoxylin	Yes	Contains no mercury, no formalin, no PVA; does not require albumin for adhesion

[a] EIA, enzyme immunoassay; FA, fluorescent antibody; MIF, merthiolate-iodine-formalin; ND, no data; PVA, polyvinyl alcohol; SAF, sodium acetate-acetic acid-formalin.

[b] Fecal immunoassays for the *Entamoeba histolytica/E. dispar* group or the true pathogen, *E. histolytica*, require fresh or frozen specimens; in some cases Cary-Blair may be acceptable—check with the manufacturer.

[c] These two fixatives use the mercuric chloride base in the Schaudinn's fluid; this formulation is still considered to be the "gold standard" against which all other fixatives are evaluated (organism morphology after permanent staining). Additional fixatives prepared with nonmercuric chloride-based compounds are used; however, the overall organism morphology is not as good.

[d] This modification uses a copper sulfate base rather than mercuric chloride; organism morphology is marginal.

[e] This modification uses a zinc base rather than mercuric chloride and works well with both trichrome and iron hematoxylin stains.

[f] These modifications use a combination of ingredients (including zinc), but are prepared from proprietary formulas. The aim is to provide a fixative that can be used for the fecal concentration, permanent stained smear, and available immunoassays for *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*), *Cryptosporidium* spp., and *Entamoeba histolytica* (or the *Entamoeba histolytica/E. dispar* group).

[g] Universal Fixatives contain no mercury, no formalin, and no PVA. The complete O&P (concentration, permanent stained smear), fecal immunoassays for *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) and *Cryptosporidium* spp., and special stains for the coccidia and microsporidia can be performed using these options. Fecal immunoassays for the *Entamoeba histolytica/E. dispar* group or the true pathogen *E. histolytica* require fresh or frozen specimens; in some cases Cary-Blair may be acceptable—check with the manufacturer. An example would be TOTAL-FIX (Medical-Chemical Corporation, Torrance, CA). One Universal Fixative (TOTAL-FIX) has also been found to be compatible with a number of molecular test options, including PCR.

vial. Fecal mmunoassays for the Entamoeba histolytica/E. dispar *group and* Entamoeba histolytica *are limited to fresh or frozen fecal specimens (some kits may work with stool submitted in Cary-Blair; check the package insert).* After centrifugation, special stains for the coccidia (modified acid-fast stains) and the microsporidia (modified trichrome stains) can be performed from the concentrate sediment obtained from formalin-preserved stool material. The most common formalin preparation is 10% formalin, prepared as follows:

Formaldehyde (USP) (or 50 ml 5%)	100 ml
Saline solution, 0.85% NaCl (or 950 ml 5%)	900 ml

Dilute 100 ml of formaldehyde with 900 ml of 0.85% NaCl solution. (Distilled water may be used instead of saline solution.)

Note Formaldehyde is normally purchased as a 37 to 40% HCHO solution; however, for dilution, it should be considered to be 100%.

If you want to use buffered formalin, the recommended approach (1, 3) is to mix thoroughly 6.10 g of Na_2HPO_4 and 0.15 g of NaH_2PO_4 and store the dry mixture in a tightly closed bottle. Prepare 1 liter of either 10 or 5% formalin, and add 0.8 g of the buffer salt mixture.

Protozoan cysts (not trophozoites), coccidian oocysts, microsporidian spores, helminth eggs, and larvae are well preserved for long periods in 10% aqueous formalin. Hot (60°C) formalin can be used for specimens containing helminth eggs, since in room-temperature formalin, some thick-shelled eggs (e.g., *Ascaris lumbricoides*) continue to develop, become infective, and remain viable for long periods. Several grams of fecal material should be thoroughly mixed in 5 or 10% formalin.

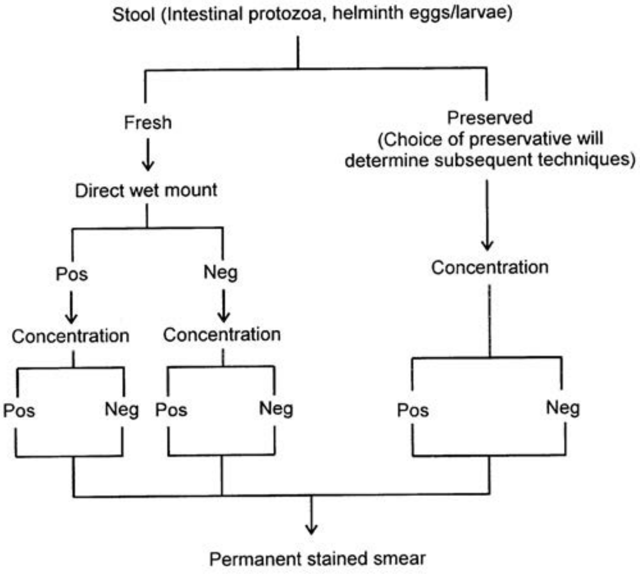

Stool (Intestinal protozoa, helminth eggs/larvae)
Fresh
Preserved
(Choice of preservative will determine subsequent techniques)
Direct wet mount
Pos
Neg
Concentration
Concentration
Concentration
Pos
Neg
Pos
Neg
Pos
Neg
Permanent stained smear

Figure 2.3 Flow diagram for preservation and processing of stool specimens. As mentioned in the text, the examination of fecal specimens using the ova and parasite examination is not considered complete unless a concentration and a permanent stained smear are examined for every specimen submitted to the laboratory. For a fresh specimen, a direct wet mount should be performed if the specimen is very soft to liquid; the complete ova and parasite examination would include the direct wet mount, the concentration, and the permanent stained smear. If the specimen is submitted in preservative, the direct wet mount should be eliminated (no motility is possible); the complete ova and parasite examination would include the concentration and the permanent stained smear (1, 2). doi:10.1128/9781555819002.ch2.f2.3

To collect large numbers of cysts, eggs, or larvae relatively free from other debris, the whole stool specimen is mixed in water and then strained through several layers of gauze. The suspension is allowed to sediment in a cone-shaped glass or flask for 1 h or more, and the supernatant fluid is discarded. The specimen may be washed several times in this manner before the sediment is finally fixed in hot 10% formalin, as mentioned above. When working with watery diarrhea specimens from patients with suspected cases of coccidiosis or microsporidiosis, the specimen should not be strained through gauze (oocysts and small bits of mucus may cling to the gauze); centrifugation (500 × *g* for 10 min) is necessary to sediment the oocysts and/or spores.

SUMMARY: Formalin

Advantages	Disadvantages
Good overall fixative for stool concentration	Does not preserve trophozoites well; must be used with a second vial for the permanent stain
Easy to prepare, long shelf life	Does not adequately preserve organism morphology for a good permanent stained smear
Formalinized stool can be used with some of the immunoassay detection kits (*Giardia lamblia*, *Cryptosporidium* spp.)	Many laboratories are trying to eliminate formalin entirely from the microbiology section

MIF

MIF (5) is a good stain preservative for most kinds and stages of parasites found in feces; it is especially useful for field surveys. It is used with all common types of stools and aspirates; protozoa, eggs, and larvae can be diagnosed without further staining in temporary wet mounts, either

Figure 2.4 Stool collection vial containing 10% formalin; note the "FILL LINE" and the poison signs at each side of the label. When the stool specimen is added to the vial, the final ratio of stool to preservative is about 1:3. doi:10.1128/9781555819002.ch2.f2.4

MCC
575-
10% FORMALIN FIXATIVE
For Ova and Parasite Detection
Contains Formaldehyde
Potential Cancer Hazard
FILL LINE
POISON / DO NOT DRINK
VENENOSO / NO BEBER
POISON / NE PAS BOIRE
GIFT / NICHT TRINKEN
If Ingested Contact a Physician Immediately.
5907 EXP05-0
For Invitro Diagnostic Use
15ml
Store at Room Temp
Chemical Corp.
Santa Monica

made immediately after fixation or prepared several weeks later. Although some laboratories maintain that a permanent stained smear can be prepared from specimens preserved in MIF, most laboratories using such a fixative examine the material only as a wet preparation (direct smear and/or concentration sediment). For a good discussion of this technique, see reference 27.

The MIF preservative is prepared in two stock solutions, stored separately, and mixed immediately before use.

Solution I (stored in a brown bottle)

Distilled water	50 ml
Formaldehyde (USP)	5 ml
Thimerosal (tincture of Merthiolate, 1:1,000)	40 ml
Glycerin	1 ml

Solution II (Lugol's Solution) (good for several weeks in a tightly stoppered brown bottle)

Distilled water	100 ml
Potassium iodide crystals (KI)	10 g
Iodine crystals (add after KI dissolves)	5 g

Combine 9.4 ml of solution I with 0.6 ml of solution II just before use.

1. Add about one-quarter teaspoon (1 g) of fresh feces to the solution, and mix with an applicator stick. Fecal material should be formed or soft if egg counts are to be made later; liquid stool does not work very well for worm burden estimates.
2. Within 24 h, if undisturbed, the specimen forms three well-defined layers. The top layer, a clear orange fluid, consists mainly of formalin, Merthiolate, and water; it does not trap eggs or protozoa. The interface is a thick, pale orange or creamy yellow layer, usually 1 to 2 mm thick; this layer may trap some protozoa and helminth eggs. The bottom layer consists of deeper-staining particulate matter; eggs and protozoa are found throughout this layer.
3. With a glass pipette, MIF direct smears can be made from both the interface and bottom layers. Best results are obtained by making smears from both layers.
4. It has been suggested by some workers that a concentration technique applied to the MIF method (referred to as the MIFC or TFC method) gives satisfactory results. This contention is debatable, and Dunn (27) suggests that it is not nearly as reliable as the MIF direct smear method.

SUMMARY: MIF

Advantages	Disadvantages
Components both fix and provide stain color	Contains mercury compounds (thimerosal); disposal a problem
Easy to prepare, long shelf life	Morphology of organisms on permanent stained smears generally not as good as that seen with Schaudinn's fluid or other fixatives (zinc-based, Universal Fixative)
Very useful for field surveys	Rarely used in majority of laboratories

SAF

SAF lends itself to the concentration technique, the permanent stained smear, and fecal immunoassays for *Giardia* and *Cryptosporidium* and has the advantage of not containing mercuric chloride, as is found in Schaudinn's fluid and some of the other fixatives containing PVA (20, 21). It is a liquid fixative, much like the 10% formalin described above (Fig. 2.5). The sediment is used to prepare the permanent smear, and it is frequently recommended that the stool material be placed on an albumin-coated slide to improve adherence to the glass (Fig. 2.6).

SAF is considered to be a "softer" fixative than mercuric chloride. The organism morphology is not quite as

Figure 2.5 Stool collection vials, one containing SAF fixative and the other containing Z-PVA, one of the non-mercury-based fixatives. This combination of collection vials is an excellent option; concentrations and fecal immunoassays can be performed from the SAF vial, while the permanent stained smear can be performed from the Z-PVA vial.
doi:10.1128/9781555819002.ch2.f2.5

MCC
SAF FIXATIVE
FILL LINE
POISON / DO NOT DRINK
VENENOSO / NO BEBER
POISON / NE PAS BOIRE
EXP
MCC
Z-PVA FIXATIVE
contains no mercury
FILL LINE
POISON / DO NOT DRINK
VENENOSO / NO BEBER
POISON / NE PAS BOIRE
EXP

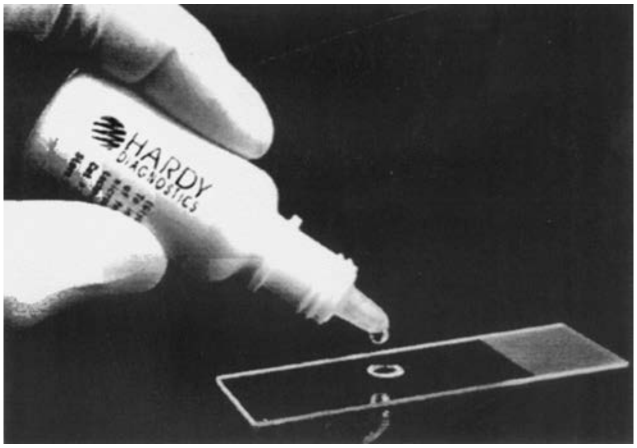


Figure 2.6 Albumin used to precoat the slide prior to the application of SAF-fixed stool concentration sediment. Once the smear is dry, it is ready for permanent staining.
doi:10.1128/9781555819002.ch2.f2.6

sharp after permanent staining as that of organisms originally fixed in solutions containing mercuric chloride. *The pairing of SAF-fixed material with iron hematoxylin staining provides better organism morphology than does staining SAF-fixed material with trichrome (personal observation)*. However, a change in the trichrome formulation tends to improve the trichrome staining using SAF (see chapter 3 on examination of fecal specimens). Although SAF has a long shelf life and is easy to prepare, the smear preparation technique may be a bit more difficult for less experienced laboratory personnel who are not familiar with fecal specimen techniques. Laboratories that have considered using only a single preservative have selected this option (concentration, permanent stain, fecal immunoassays for *Giardia* and *Cryptosporidium*). Helminth eggs and larvae, protozoan trophozoites and cysts, coccidian oocysts, and microsporidian spores are preserved by this method. After centrifugation, special stains for the coccidia (modified acid-fast stains) and the microsporidia (modified trichrome stains) can be used with the concentrate sediment obtained from SAF-preserved stool material.

SAF fixative is prepared as follows:

Sodium acetate	1.5 g
Acetic acid, glacial	2.0 ml
Formaldehyde, 37 to 40% solution	4.0 ml
Distilled water	92.0 ml

To make Mayer's albumin, mix equal parts of egg white and glycerin. Place 1 drop on a microscope slide, and add 1 drop of SAF-preserved fecal sediment (from the concentration procedure). After mixing, allow the smear to dry at room temperature for 30 min prior to staining. Mayer's albumin is also available commercially.

SUMMARY: SAF

Advantages	Disadvantages
Can be used for concentration and permanent stained smears	Adhesive properties may cause problems; albumin-coated slides an option
Contains no mercury compounds	More commonly stained using iron hematoxylin; modified trichrome formula enhances staining of SAF-preserved specimens (see chapter 3 on examination of fecal specimens)
Easy to prepare, long shelf life	May be a bit more difficult to use; however, this does not seem to be a limiting factor
SAF-preserved stool can be used with immunoassay methods (*Giardia* and *Cryptosporidium*)	Contains formalin; some laboratories are trying to remove formalin from the microbiology section
One-vial system; less costly	

Schaudinn's Fluid

Schaudinn's fluid is designed to be used with fresh stool specimens or samples from the intestinal mucosal surface. Many laboratories that receive specimens from in-house patients (no problem with delivery times) often select this approach. Permanent stained smears are then prepared from fixed material. A concentration technique for Schaudinn's fluid-preserved material is also available but is not widely used.

Mercuric Chloride, Saturated Aqueous Solution

Mercuric chloride ($HgCl_2$)	110 g
Distilled water	1,000 ml

Use a beaker as a water bath; boil (use a hood if available) until the mercuric chloride is dissolved; let stand for several hours until crystals form.

Schaudinn's Fixative (Stock Solution)

Mercuric chloride, saturated aqueous solution	600 ml
Ethyl alcohol, 95%	300 ml

Immediately before use, add 5 ml of glacial acetic acid per 100 ml of stock solution.

SUMMARY: Schaudinn's fluid

Advantages	Disadvantages
Fixative for smears prepared from fresh fecal specimens or samples from the intestinal mucosal surfaces	Not generally recommended for use in concentration procedures
Provides excellent preservation of protozoan trophozoites and cysts	Contains mercuric chloride—disposal problem
For many years, considered the "gold standard"	Poor adhesive qualities with liquid or mucoid specimens

Schaudinn's fluid containing PVA (mercury base)

PVA is a plastic resin that is normally incorporated into Schaudinn's fixative (28). The PVA powder is inert and is *not* a fixative but serves as an adhesive for the stool material; i.e., when the stool-PVA-fixative mixture is spread onto the glass slide, it adheres because of the PVA component. Fixation is still accomplished by the Schaudinn's fluid itself. Perhaps the greatest advantage of the use of PVA is the fact that a permanent stained smear can be prepared. Although some laboratories may perform a fecal concentration from a PVA-preserved specimen, some parasites do not concentrate well, nor do some exhibit the typical morphology that would be seen in concentration sediment from a formalin-based fixative. Fixatives containing PVA are highly recommended as a means of preserving cysts and trophozoites for later examination. The use of fixatives containing PVA also permits specimens to be shipped (by regular mail service) from any location in the world to a laboratory for subsequent examination. Fixatives containing PVA are particularly useful for liquid specimens and should be used in the ratio of 3 parts PVA to 1 part fecal specimen. The formula is as follows:

PVA	10.0 g
Ethyl alcohol, 95%	62.5 ml
Mercuric chloride, saturated aqueous	125.0 ml
Acetic acid, glacial	10.0 ml
Glycerin	3.0 ml

Mix the liquid ingredients in a 500-ml beaker. Add the PVA powder (stirring is not recommended). Cover the beaker with a large petri dish, heavy wax paper, or foil, and allow the PVA to soak overnight. Heat the solution slowly to 75°C. When this temperature is reached, remove the beaker and swirl the mixture for 30 s until a homogeneous, slightly milky solution is obtained.

SUMMARY: Schaudinn's fluid containing PVA (mercury base)

Advantages	Disadvantages
Can be used to prepare permanent stained smears and perform concentration techniques (see also Disadvantages)	*Trichuris trichiura* eggs and *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) cysts are not concentrated as easily as from formalin-based fixatives; *Strongyloides stercoralis* larval morphology is poor (better to use formalin-based preservation); *Cystoisospora* (*Isospora*) *belli* oocysts may not be visible in PVA fixative-preserved material (better to use formalin-fixed specimens); usually combined with a formalin collection vial
Provides excellent preservation of protozoan trophozoites and cysts	Contains mercury compounds (Schaudinn's fluid)
Long shelf life (months to years) in tightly sealed containers at room temperature	May turn white and gelatinous when it begins to dehydrate or when refrigerated
Allows specimens to be shipped to any laboratory for subsequent examination	Difficult to prepare in the laboratory
Specimens preserved in PVA cannot be used with the fecal immunoassay kits	For many reasons, this fixative is being phased out in most laboratories

Schaudinn's fluid containing PVA (copper base, zinc base)

There has been a great deal of interest in developing preservatives without the use of mercury compounds, and substitute compounds now provide the quality of preservation necessary for comparable protozoan morphology on the permanent stained smear. Copper sulfate has been tried (29, 30) but does not provide results equal to those seen with mercuric chloride (29). However, zinc sulfate has proven to be a good mercury substitute and is used with trichrome stain or iron hematoxylin (Fig. 2.5) (31, 32). Although zinc substitutes have become widely available, each manufacturer has a proprietary formula for the fixative.

Copper Sulfate Solution

$CuSO_4 \cdot 5H_2O$	20.0 g
Distilled water	1,000 ml

Add the $CuSO_4 \cdot 5H_2O$ to 1,000 ml of distilled water heated to 100°C. Mix until dissolved.

Modified PVA Fixative (Stock Solution)

Copper sulfate solution	600 ml
Ethyl alcohol, 95%	300 ml

Immediately before use, add 5 ml of glacial acetic acid per 100 ml of stock solution.

SUMMARY: Schaudinn's fluid containing PVA (copper base, zinc base)

Advantages	Disadvantages
Can prepare permanent stained smears and perform concentration techniques	Overall protozoan morphology of trophozoites and cysts is poor when preserved in the copper sulfate-based fixative, particularly compared with organisms preserved with mercuric chloride-based fixatives

(continued)

SUMMARY: Schaudinn's fluid containing PVA (copper base, zinc base) *(continued)*

Advantages	Disadvantages
Many workers prefer the zinc substitutes over those prepared with copper sulfate	Zinc-based fixatives are a better alternative
Does not contain mercury compounds	Staining characteristics of protozoa not consistent—some good, some poor; organism identification may be more difficult, particularly with small protozoan cysts

Single-Vial Collection Systems (Other than SAF)

Several manufacturers now have available single-vial stool collection systems, similar to SAF or modified PVA methods (11). From the single vial, both the concentration and permanent stained smear can be prepared (Fig. 2.7 through 2.9). It is also possible to perform fecal immunoassay procedures from some of these vials. Make sure to ask the manufacturer about all three capabilities (concentration, permanent stained smear, fecal immunoassay procedures) and for specific information indicating that there are no formula components that would interfere with any of the three methods. Like the zinc substitutes, these formulas are proprietary.

Figure 2.7 ECOFIX is an example of a fixative that represents the single-vial collection system. This fixative has been coupled with its own stain, the ECOSTAIN (11). Remember to inquire about the compatibility of all single-vial systems with the immunoassay procedures; not all single-vial preservatives are compatible with all fecal immunoassays.
doi:10.1128/9781555819002.ch2.f2.7

Para-Pak
ECOFIX™
ADD SPECIMEN TO THIS LINE
POISON/DON'T DRINK
VENENOSO/NO BEBER
POISON/NE BUVEZ PAS
GIFT/NICHT TRINKEN

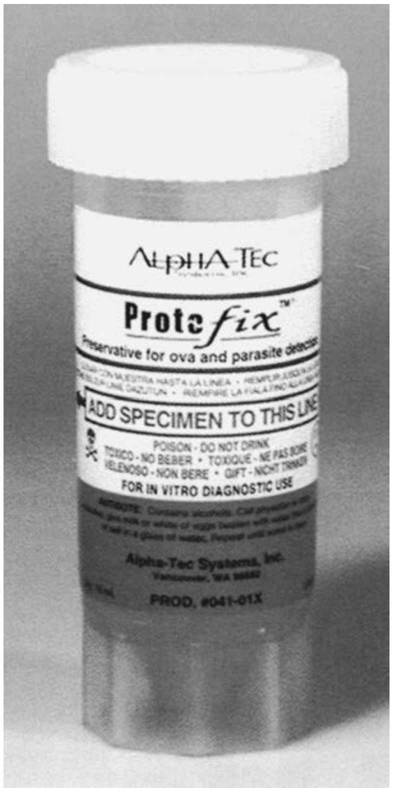
SUMMARY: Single-vial collection systems (other than SAF)

Advantages	Disadvantages
Can be used to prepare permanent stained smears and perform concentration techniques	Overall protozoan morphology of trophozoites and cysts is not as good as that for organisms preserved with mercuric chloride-based fixatives; morphology similar to modified PVA options (zinc base)
Can perform most tests from this single vial, PROVIDING IT DOES NOT CONTAIN PVA	Important to review package inserts for information on using for fecal immunoassays

Universal Fixative (TOTAL-FIX)

The TOTAL-FIX stool collection kit is a single-vial system that provides a standardized method for untrained personnel to properly collect and preserve stool specimens for the detection of helminth larvae and eggs, protozoan trophozo-

Figure 2.8 ProtoFix is a single-vial collection option. It is always important to review peer-reviewed literature regarding the results of new products compared with those previously in use.
doi:10.1128/9781555819002.ch2.f2.8


ALPHA-TEC
Protofix™
Preservative for ova and parasite detection
ADD SPECIMEN TO THIS LINE
POISON - DO NOT DRINK
FOR IN VITRO DIAGNOSTIC USE
Alpha-Tec Systems, Inc.
PROD. #041-01X

ites and cysts, coccidian oocysts, and microsporidian spores. Concentrations, permanent stains, most fecal immunoassays, and some molecular methods can be performed from a TOTAL-FIX preserved specimen (Fig 2.10). TOTAL-FIX is a mercury-, formalin-, and PVA-free fixative that preserves parasite morphology and helps with disposal and monitoring problems encountered by laboratories. www.med-chem.com (accessed 4/17/13). TOTAL-FIX is similar to Unifix and Zinc PVA (Z-PVA), commonly used fixatives that have been commercially available and used in many laboratories since 1992.

SUMMARY: Universal Fixative (TOTAL-FIX)

Advantages	Disadvantages
Can prepare permanent stained smears and perform concentration techniques	Organism morphology will not always equal the mercuric chloride "gold standard"—however, for the most part organism morphology is comparable
Overall protozoan morphology of trophozoites and cysts is quite good; in some cases, equals results using mercuric chloride-based fixatives	
Can perform fecal immunoassays (EIA, FA, rapid cartridges) and special stains for coccidia and microsporidia (modified acid-fast, modified trichrome)	
Does not contain mercury compounds, does not contain formalin, does not require PVA or albumin for adhesive	
Can be used for some molecular testing—may vary	
Single-vial system; less costly than the two-vial approach	

MCC
UNIFIX™
For Ova and Parasite Detection
Mercury and Formalin Free
FILL LINE
POISON / DO NOT DRINK
VENENOSO / NO BEBER
POISON / NE PAS BOIRE
GIFT / NICHT TRINKEN
If Ingested Contact a Physician Immediately
LOT 4087 EXP 07-
For Invitro Diagnostic Use
Contains 15ml Store at Room Temp
Medical Chemical Corp. Santa

Figure 2.9 UNIFIX is a single-vial collection system; information on this product can be found at the company website (www.med-chem.com). This fixative works well with trichrome stain. doi:10.1128/9781555819002.ch2.f2.9

Use of Fixatives

Quality Control for Stool Fixatives

Fixatives for fecal specimens are checked for quality control by the manufacturer before sale, generally with the use of living protozoa. If you prepare your own fixatives, the following approach can be used for quality control. The specimen used for quality control presented below is designed to be used with fixatives from which permanent stained smears will be prepared (Schaudinn's fluid, Schaudinn's fluid containing PVA, copper- or zinc-based fixative containing PVA, SAF, MIF, and Universal Fixatives). However, the same quality control specimen can also be used in a concentration; the white blood cells (WBCs) can be seen in the concentrate sediment (sedimentation concentration) or in the surface film (flotation concentration).

1. Obtain a fresh, anticoagulated blood specimen, centrifuge, and obtain a buffy coat sample (try and find a specimen with a high WBC count).
2. Mix approximately 2 g of soft, fresh fecal specimen (normal stool, containing no parasites) with several drops of the buffy coat cells.
3. Prepare several fecal smears, and fix immediately in Schaudinn's fluid to be quality controlled.
4. Mix the remaining feces-buffy coat mixture in 10 ml of fixatives with or without PVA, SAF, MIF, or Universal Fixative preservative to be quality controlled.
5. Allow 30 min for fixation, and then prepare several fecal smears. Allow to dry thoroughly (60 min at room temperature or 30 to 60 min in an incubator [approximately 35°C]). Do not use a heat block.
6. Stain the slides by the normal staining procedure (trichrome, iron hematoxylin).
7. After staining, if the WBCs appear well fixed and display typical morphology and color, one can assume that any intestinal protozoa placed in the same lot number of preservative would also be well fixed, provided that the fecal sample was fresh and fixed within the recommended time limits.
8. The bulk quality control specimen can be concentrated as for a normal patient specimen. If the fixative is performing correctly, the WBCs will be visi-

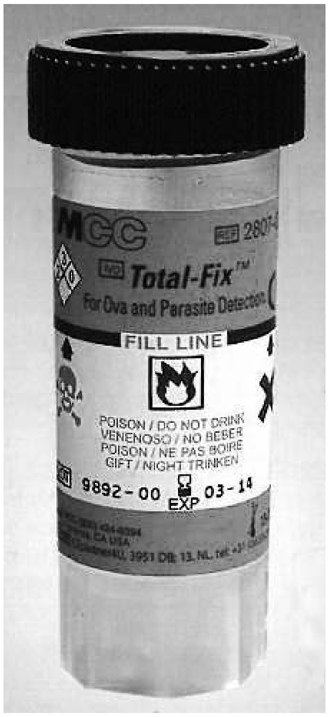


Figure 2.10 TOTAL-FIX is a single-vial collection system; the fixative contains no mercury, no formalin, and no PVA. It is considered a Universal Fixative and can be used for concentrations, permanent stained smears, fecal immunoassays, special stains, and some molecular methods.
doi:10.1128/9781555819002.ch2.f2.10

ble in the concentration sediment or surface film (depending on the method used).

9. Record all quality control results. If the WBC morphology does not confirm good fixation, describe the results and indicate what corrective actions were used (repeated the test, prepared new fixative).

Procedure Notes for Use of Preservatives

1. Most of the commercially available kits have a "fill to" line on the vial label to indicate how much fecal material should be added to ensure adequate preservation of the fecal material (ratio of 1 part stool to 3 parts fixative). *However, patients often overfill the vials; remember to open the vials with the vials turned away from your face. There may be excess gas in the vials that may create aerosols once the vial lids are opened.*
2. Although the two-vial system (one vial of 5 or 10% buffered formalin [concentration] and one vial of PVA fixative [permanent stained smear]) has always been the "gold standard," many laboratories are now using other options, including the single-vial collection systems and the Universal Fixative. Changes in the selection of fixatives are based on the following considerations:
   - A. Problems with disposal of mercury-based fixatives (availability of high-temperature incineration facilities and cost) and lack of multilaboratory contracts for disposal of such products
   - B. The cost of a two-vial system compared with the cost of a single collection vial
   - C. Selection of specific stains (trichrome, iron hematoxylin) to use with specific fixatives
   - D. Whether the newer fecal immunoassay kits can be used with stool specimens preserved in that particular fixative

Procedure Limitations for Use of Preservatives

1. Adequate fixation still depends on the following parameters:
   - A. Meeting recommended time limits for lag time between passage of the specimen and fixation
   - B. Use of the correct ratio of specimen to fixative (1:3)
   - C. Thorough mixing of the fixative and specimen (once the specimen is received in the laboratory, any additional mixing at that time will not counteract the earlier lack of fixative-specimen mixing and contact)
2. Unless the appropriate stain is used with each fixative, the final permanent stained smear may be difficult to examine (organisms hard to see and/or identify). Examples of appropriate combinations are as follows:
   - A. Schaudinn's or PVA fixative with trichrome or iron hematoxylin stain
   - B. SAF fixative with iron hematoxylin stain or the modified trichrome formulation

Shipment of Diagnostic Specimens, Biological Products, Etiologic Agents, or Infectious Substances

Biological materials shipped domestically or internationally must be packaged in compliance with hazardous-materials transport regulations (33, 34). The United States has incorporated the United Nations Recommendations on the Transport of Dangerous Goods into law, making the U.S. regulations consistent with foreign shipment regulations. The U.S. regulations for packing diagnostic specimens and infectious agents for shipment were modified in 2006. The Public Health Service, Department of Transportation, and Postal Service specify requirements for packaging and shipping of biological materials. These regulations, plus those for packaging and shipping of materials via air, are similar;

TABLE 2.5 Agencies governing transportation of dangerous goods

Governing authority	Agency[a]	Regulations
United Nations	ICAO	*Technical Instructions for the Safe Transport of Dangerous Goods by Air* http://www.icao.int/about-icao/Pages/default.aspx (accessed 4/18/13)
Commercial airline industry	IATA (also known as ICAO)	*Dangerous Goods Regulations* http://www.iata.org/whatwedo/cargo/dgr/Pages/index.aspx (accessed 4/18/13) IATA requirements have become the most widely recognized, copied, and used packing and shipping guidelines in the world.
United States	DOT (shipments using ground transportation in the United States)	*United States Hazardous Materials Uniform Safety Act* http://www.dot.gov/regulations (accessed 4/18/13) In the United States, the DOT regulates the commercial transportation of dangerous goods by both air and ground carriers. Just as IATA derives its requirements from ICAO, the DOT also derives its regulations from ICAO.
United States	USPS	*Domestic Mail Manual. CO23 Hazardous Materials* http://pe.usps.com/text/pub52/welcome.htm (accessed 4/18/13)
Canada	Transport Canada	*Transportation of Dangerous Goods Regulations* http://www.tc.gc.ca/eng/tdg/safety-menu.htm (accessed 4/18/13)
Other nations		Individual national regulations
World	IMDG (shipments using ocean vessel) transportation	*Transportation of Dangerous Goods: International Maritime Dangerous Goods Code* http://phmsa.dot.gov/hazmat/regs/sp-a/approvals/imdg-icao (accessed 4/18/13)

[a] ICAO, International Civil Aviation Organization; IATA, International Air Transport Association; DOT, Department of Transportation; USPS, U.S. Postal Service; IMDG, International Maritime Dangerous Goods.

most carriers elect to follow the international shipping regulations within the International Air Transport Association (IATA) *Dangerous Goods Regulations* (Table 2.5).

Anyone packaging diagnostic specimens or infectious agents for shipment must receive training and be tested every 2 years. Those who complete the training and test will receive a certificate. The regulations are designed to protect all personnel who may come in contact with the package; compliance with these rules is the responsibility of the sender. Definitions of relevant terms can be found in Tables 2.5 and 2.6.

Double mailing containers should be used in shipping any parasitologic specimens other than microscope slides. The inner container is an aluminum screw-cap mailing tube that fits into an outer cardboard screw-cap mailing container. The specimen vials or tubes in the inner aluminum cylinder should be packed in cotton to absorb any moisture or material that might result from leakage or breakage. Instruction sheets, patient information sheets, etc., may be wrapped around the metal cylinder before it is placed in the outer cardboard mailer. For all packages containing infectious substances, an itemized list of contents must be enclosed between the secondary packaging and the outer packaging (33, 34). Training and training material for the transportation of dangerous goods and infectious substances are available at the American Society for Microbiology and IATA (training manuals).

Prepared slides, such as stained fecal smears or blood films, do not require double mailers for shipment. They may be packed in boxes, cardboard slide holders, or any other suitable container that will prevent damage or breakage. Slides should be individually wrapped in toilet tissue or facial tissue. If a number of slides are to be mailed, they can be wrapped in toilet paper as follows: place a slide on the tissue, wrap the slide several times, place the next slide on top of the first, and continue to wrap the slide several times. The series of slides will be padded and can be easily unwrapped on arrival. When you place slides in the flat cardboard containers, they need additional protection; some slides will arrive broken if this thin cardboard con-

TABLE 2.6 Definitions of relevant terms for packing and shipping[a]

Term	Definition
Biological product	A substance which originated from living organisms (including humans and other mammals) and has been manufactured and distributed in accordance with compliance and licensing requirements set forth by the federal government; can be classified as an infectious substance or a diagnostic (or clinical) specimen if such is appropriate. Biological products can be finished or unfinished; are intended for use in the prevention, treatment, or diagnosis of disease in humans or animals; and can be used for investigational, experimental, or development purposes. Biological products include such common items as clinical microbiology reagents and kits, serologic reagents, diagnostic reagents, and vaccines. In certain parts of the world, some licensed biological products are regarded as biohazardous and either are subject to compliance criteria specified for infectious substances or must adhere to other restrictions imposed by the government of that country.
Biological substance, Category B	Any infectious substance which does not meet the criteria of a Category A substance; formerly known as *clinical specimen* or *diagnostic specimen*; an infectious substance *not* in a form generally capable of causing disability, life-threatening illness, or fatal disease. Category B substances generally are (i) patient and clinical specimens reasonably expected to contain, or being cultured or otherwise tested for a non-Category A pathogen and (ii) cultures of microorganisms not specifically listed in Category A. The proper term for a Category B substance is Biological Substance, Category B.
Carrier (operator)	Individual or organization engaged in the commercial transportation of goods (e.g., DHL, Federal Express, United Parcel Service, Delta Airlines, and Northwest Airlines).
Category A substance	An infectious substance or microorganism that is transported *in a form* which, when exposure to it occurs, is capable of causing permanent disability or is life-threatening.
Category B substance	An infectious substance which does not meet Category A criteria; an infectious substance *not* in a form capable of causing disability, life-threatening illness, or a fatal disease. Category B substances generally are considered to be (i) patient or clinical specimens reasonably expected to contain, or being cultured or otherwise tested for, a non-Category A pathogen and (ii) cultures of microorganisms not specifically listed in Category A.
Code of Federal Regulations (CFR)	U.S. laws published in the *Federal Register* and available online at http://www.gpoaccess.gov/cfr/index.html (accessed 4/18/13)
Culture	The result of a process by which pathogens are intentionally propagated. This definition refers to typical laboratory cultures of microorganisms grown in broth or on solid media. Typical clinical cultures may be classified as either Category A or Category B, depending on the organism concerned and the professional judgment of the shipper.
Dangerous goods	Materials which, when not properly handled and contained, can pose a risk to the health, safety, property, or environment and which are shown on the list of dangerous goods in the IATA *Dangerous Goods Regulations*. http://www.iata.org/whatwedo/cargo/dgr/Pages/index.aspx (accessed 4/18/13)
Diagnostic or clinical specimen	Term no longer used or allowed; replaced by "Biological Substance, Category B"
Infectious substance	A substance which is known or reasonably expected to contain pathogens (microorganisms which can cause disease in humans and animals); material known to contain or reasonably suspected of containing a Category A or B pathogen or substance; can be a class (class 6), a division (division 6.2), or a category (Category A or B) of dangerous goods as defined by IATA.
Packaging	All of the materials used to contain a shipped substance and to prepare the substance for shipping: the container (receptacle) and its associated components (e.g., tubes, containers, absorbent material, boxes, and labels) used to contain and pack a substance and to ensure compliance with packing requirements.
Packing instructions	IATA-defined directions that shippers must follow to select, assemble, mark, label, and document the packing process for shipping dangerous goods, including diagnostic specimens and infectious substances; include manufacturing, testing, and performance specifications for packaging materials. **NOTE:** On January 1, 2011, packing instructions (PI) 602, 904, and 913 were renumbered to 620, 954, and 959, respectively.
Pathogen	A microorganism (bacterium, mycobacterium, fungus, parasite, virus, plasmid, genetic element, proteinaceous infectious particle [prion], or genetically modified organism) that is known to cause or is reasonably expected to be able to cause disease in humans or animals.
Patient specimen	Material collected from humans or animals, including but not limited to excreta, secreta, blood and its components, tissue, body fluids, body organs and parts, and swabs of human material, being transported for purposes such as research, diagnosis, investigational activities, and disease treatment and prevention.
Primary specimen container	The innermost packaging containing a diagnostic specimen or infectious substance; composed of glass, metal, or plastic; must be leakproof; must be positively sealed if it contains an infectious substance.
Secondary specimen container	The container that contains the primary specimen container.
Shipper	Anyone who ships goods by a commercial carrier (usually an employee of a company or health care facility [e.g., laboratory staff member]); anyone who offers goods for transport to a member of IATA; anyone who completes and signs the Shipper's Declaration. The person who signs the Shipper's Declaration is the person who accepts responsibility for the accuracy of the information on the document.
Shipper's Declaration for Dangerous Goods	An IATA-defined and -mandated form which must accompany each shipment of dangerous goods; contains information that describes the dangerous goods; is helpful to persons who handle the shipment; must be completed by the shipper.

[a] Adapted from reference 34 and http://who.int/ihr/biosafety/module_ii_categorization.pdf (accessed 22 May 2015).

tainer is merely placed in an envelope for mailing. A plastic slide container with a snap top is an excellent option for shipping microscope slides (holds four to five slides).

Note If the slides are mounted with Permount or other mounting media, they should be completely dry before being packed.

All packages prepared for mailing should contain a complete information sheet about the specimen. An address label should be enclosed inside the package, as well as being on the outside container. All labels should be checked to ensure proper delivery. Also, remember that if you use an overnight carrier, the address must also contain a street address, not just a P.O. Box or general address such as department and institution.

Documentation

The Shipper's Declaration for Dangerous Goods is a legal document that serves as a contract between the shipper and carrier; it must be accurate and legible and have no spelling errors. However, if minor discrepancies in typing or punctuation are present, they are no longer considered errors if they do not compromise safety. The document must be either handwritten by one person or typed. Two copies of the Shipper's Declaration must be completed and signed for each shipment. One copy is retained by the shipper, and the other is forwarded with the package; one copy can be a carbon copy. This document is required for shipping infectious substances and toxic substances; it is not required for shipping diagnostic specimens or biological products. If there are no dangerous goods in a shipment with dry ice, the Shipper's Declaration is not required. It is important for personnel within the laboratory to thoroughly understand these regulations and to have sample copies of appropriate documentation for referral. A checklist used by carriers to decide to accept or reject a package is widely used; a copy of this document should also be on file within the laboratory.

The current editions of the Code of Federal Regulations can be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402. Inquiries about Postal Service publications may be directed to U.S. Postal Service, 475 L'Enfant Plaza, Washington, DC 20260-5365. Dangerous Goods Regulations can be obtained from Publications Assistant, International Air Transport Association, 2000 Peel Street, Montreal, Quebec, Canada, H3A 2R4.

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3

Macroscopic and Microscopic Examination of Fecal Specimens

Macroscopic Examination

Microscopic Examination (Ova and Parasite Examination)

Direct wet smear

Concentration (sedimentation and flotation)

Permanent stained smear

Specialized Stains for Coccidia (*Cryptosporidium*, *Cystoisospora* [*Isospora*], and *Cyclospora* Species) and the Microsporidia

Modified Kinyoun's acid-fast stain (cold method)

Modified Ziehl-Neelsen acid-fast stain (hot method)

Carbol fuchsin negative stain for *Cryptosporidium* (from W. L. Current)

Rapid safranin method for *Cryptosporidium*

Rapid safranin method for *Cyclospora*, using a microwave oven

Auramine O stain for coccidia (from Thomas Hänscheid)

Modified trichrome stain for the microsporidia (Weber—green)

Modified trichrome stain for the microsporidia (Ryan—blue)

Modified trichrome stain for the microsporidia (Kokoskin—hot method)

Acid-fast trichrome stain for *Cryptosporidium* and the microsporidia

Macroscopic Examination

If the consistency of a stool specimen can be determined (formed, soft, or liquid), this information may give an indication of the organism stages that might be present. Trophozoites (potentially motile forms) of the intestinal protozoa are usually found in liquid specimens; both trophozoites and cysts might be found in a soft specimen; and the cyst forms are usually found in formed specimens. However, there are always exceptions to these general statements. Coccidian oocysts and microsporidian spores can be found in any type of fecal specimen; in the case of *Cryptosporidium* spp., the more liquid the stool, the more oocysts that are found in the specimen. Helminth eggs may be found in any type of specimen, although the chances of finding eggs in a liquid stool are reduced by the dilution factor. Tapeworm proglottids may be found on or beneath the stool on the bottom of the collection container. Adult pinworms and *Ascaris lumbricoides* are occasionally found on the surface or in the stool.

The presence of blood in or on the specimen may indicate several things and should always be reported. Dark stools may indicate bleeding high in the gastrointestinal tract, and fresh (bright red) blood most often is the result of bleeding at a lower level. In certain parasitic infections, blood and mucus may be present. Soft or liquid stool accompanied by blood is more suggestive of an amebic infection; these areas of blood and mucus should be carefully examined for the presence of trophic amebae. *Occult blood in the stool may or may not be related to a parasitic infection and could result from a number of different conditions.* Ingestion of various compounds may give a distinctive color to the stool (iron, black; barium, light tan to white).

Many laboratories prefer that stool specimens be submitted in some type of preservative. Rapid fixation of the specimen immediately after passage (by the patient) provides an advantage in terms of recovery and identification of intestinal protozoa. This advantage (preservation of organisms before distortion or disintegration) is thought to outweigh the limited motility information that might be gained by examining fresh specimens as direct wet mounts. Other laboratories still request a collection system that includes both a preserved specimen and the remainder of the fresh stool. Certainly cost is a factor, because several vials in the collection system cost more than a single vial containing preservative. Each

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laboratory will have to decide for itself, often basing the decision on the types of procedures ordered by the physicians who use the laboratory service, the test method selected (traditional methods, immunoassay detection kits, or molecular methods), and the lag time between specimen collection and submission to the laboratory.

With increased emphasis on continuous quality improvement, managed-care contracts, cost containment, and the clinical relevance of diagnostic test results generated, compliance with specimen acceptance or rejection criteria has become more important and a necessary part of overall quality performance. *The generation of patient data begins with the quality of the specimen; anything that is done to compromise that quality should not be acceptable within the laboratory setting.*

If the specimen has not been preserved immediately after passage, it is important to know the age of the specimen when it reaches the laboratory. Freshly passed specimens are necessary for the detection of trophic amebae, flagellates, and ciliates. Liquid specimens must be examined within 30 min of passage (not 30 min from the time the specimen reaches the laboratory or is clocked in by the computer). Soft specimens should be examined within 1 h of passage. Immediate examination of a formed specimen is not as critical; however, if the stool cannot be examined on the day of collection, portions of the specimen should be preserved. In a routine laboratory setting, these time frames are often neither practical nor possible. Thus, the routine use of stool preservatives for diagnostic parasitology is highly recommended and has been widely accepted.

Microscopic Examination (Ova and Parasite Examination)

The microscopic examination of the stool specimen, normally called the ova and parasite examination, consists of three separate techniques: the direct wet smear, the concentration, and the permanent stained smear. Each of these methods is designed for a particular purpose and forms an integral part of the total examination (1–22, 49). With increased emphasis on proper specimen collection and cost containment, the approach to the ova and parasite examination has changed somewhat during the last few years. Many laboratories are requesting that all fecal specimens be collected in preservatives prior to delivery to the laboratory to decrease the lag time between specimen passage and fixation, thus providing better organism morphology and subsequent identification.

Because preserved organisms do not exhibit motility, the direct wet smear is no longer considered a mandatory part of the routine ova and parasite examination. However, if fresh fecal specimens are delivered to the laboratory, the direct wet smear should be performed, particularly on liquid or very soft stools.

In addition to normal specimen debris, the microscopic examination of fecal material may reveal the following:

1. Trophozoites and cysts of intestinal protozoa
2. Oocysts of coccidia and spores of microsporidia
3. Helminth eggs and larvae
4. Red blood cells (RBCs), which may indicate ulceration or other hemorrhagic problems
5. White blood cells (WBCs), specifically polymorphonuclear leukocytes (PMNs), which may indicate inflammation
6. Eosinophils, which usually indicate the presence of an immune response (which may or may not be related to a parasitic infection)
7. Macrophages, which may be present in bacterial or parasitic infections
8. Charcot-Leyden crystals, which may be found when disintegrating eosinophils are present (and may or may not be related to a parasitic infection)
9. Fungi (*Candida* spp.) and other yeasts and yeastlike fungi
10. Plant cells, pollen grains, or fungal spores, which may mimic some helminth eggs, protozoan cysts, coccidian oocysts, or microsporidial spores
11. Plant fibers or root or animal hairs, which may mimic helminth larvae

Direct Wet Smear

Normal mixing in the intestinal tract usually ensures an even distribution of organisms. However, depending on the level of infection, examination of the fecal material as a direct smear may or may not reveal organisms. The direct wet smear is prepared by mixing a small amount of stool (about 2 mg) with a drop of 0.85% NaCl; this mixture provides a uniform suspension under a 22- by 22-mm coverslip. Some workers prefer a 1.5- by 3-in. (1 in. = 2.54 cm) slide for the wet preparations rather than the standard 1- by 3-in. slide, which is routinely used for the permanent stained smear. A 2-mg sample of stool forms a low cone on the end of a wooden applicator stick. If more material is used for the direct mount, the suspension is usually too thick for an accurate examination; any sample of less than 2 mg results in the examination of too thin a suspension, thus decreasing the chances of finding organisms. If present, blood or mucus should always be examined as a direct mount. The entire 22- by 22-mm coverslip should be systematically examined with the low-power objective (10×) and low light intensity (Fig. 3.1); any suspicious objects may then be examined with the high dry objective (40×).

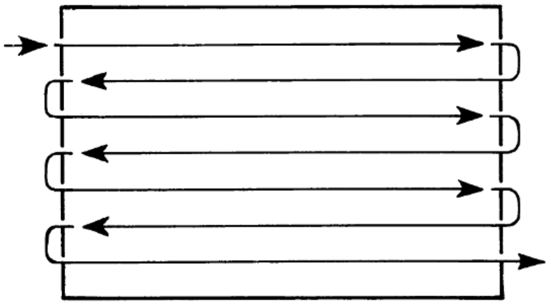

Figure 3.1 Method of scanning direct wet film preparation with a 10× objective. Note that the entire coverslip preparation should be examined before indicating the examination is negative. (Illustration by Nobuko Kitamura.) Note: All methods contained in the figures can be found in reference 48.
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At least one-third to one-half of the coverslip should be examined under high dry power (total magnification, ×400), even if nothing suspicious has been seen. Use of an oil immersion objective (100×) on mounts of this kind is not routinely recommended unless the coverslip is sealed to the slide (a no. 1 thickness coverslip is recommended for oil immersion). For a temporary seal, a cotton-tipped applicator stick dipped in equal parts of heated paraffin and petroleum jelly should be used. Nail polish can also be used to seal the coverslip. ***Many workers think that the use of the oil immersion objective on this type of preparation is impractical, especially since morphological detail is more readily seen by oil immersion examination of the permanent stained smear.*** This is particularly true in a busy clinical laboratory situation.

The direct wet mount is used primarily to detect motile protozoan trophozoites. These organisms are very pale and transparent, two characteristics that require the use of low light intensity. Protozoan organisms in a saline preparation usually appear as refractile objects. If suspicious objects are seen on high dry power, at least 15 s should be allowed to detect motility of slowly moving protozoa. Application of heat by placing a hot penny on the edge of a slide may enhance the motility of trophic protozoa. Tapping on the coverslip can also stimulate the fluid to move; objects will roll over, thus providing a better view of the parasite or artifact. Helminth eggs and/or larvae, protozoan cysts, and coccidian oocysts may also be seen on the wet film, although these forms are more likely to be detected after fecal concentration procedures (Fig. 3.2).

After the wet preparation has been thoroughly checked for trophic amebae, a drop of iodine can be placed at the edge of the coverslip or a new wet mount can be prepared with iodine alone (Fig. 3.3). A weak iodine solution is recommended; too strong a solution may obscure the organisms. Several types of iodine are available; Lugol's and D'Antoni's are discussed here. Gram's iodine, used in bacterial work, is not recommended for staining parasitic organisms.

If preserved specimens are submitted to the laboratory, it is more cost-effective and clinically relevant to omit the direct smear and begin the stool examination with the concentration procedure, particularly since motile protozoa are not viable because of the prior addition of preservative. Even if parasites are seen on a direct mount of preserved stool, they would almost certainly be seen on the concentration examination as well as on the permanent stained smear (protozoa in particular). With few exceptions, intestinal protozoa should not be identified on the basis of a wet mount alone; permanent stained smears should be examined to confirm the specific identification of suspected organisms.

Saline (0.85% NaCl)

NaCl	0.85 g
Distilled water	100.0 ml

1. Dissolve the NaCl in distilled water in a flask or bottle, using a magnetic stirrer.
2. Distribute 10 ml into each of 10 screw-cap tubes.
3. Label as 0.85% NaCl with an expiration date of 1 year.
4. Sterilize by autoclaving at 121°C for 15 min.
5. When cool, store at 4°C.

D'Antoni's Iodine

Potassium iodide	1.0 g
Powdered iodine crystals	1.5 g
Distilled water	100.0 ml

1. Using a magnetic stirrer, dissolve the potassium iodide and iodine crystals in distilled water in a flask or bottle.
2. The potassium iodide solution should be saturated with iodine, with some excess crystals left on the bottom of the bottle.
3. Store in a brown, glass-stoppered bottle at room temperature and in the dark.
4. This stock solution is ready for immediate use. Label as D'Antoni's iodine with an expiration date of 1 year (the stock solution remains good as long as an excess of iodine crystals remains on the bottom of the bottle).
5. Aliquot some of the iodine into a brown dropper bottle. The working solution should have a strong-tea color and should be discarded when the color lightens (usually within 10 to 14 days).

Note The stock and working solution formulas are identical, but the stock solution is held in the dark and will retain the strong-tea color while the working solution will fade and have to be periodically replaced (Fig. 3.4).

Lugol's Iodine

Potassium iodide	10.0 g
Powdered iodine crystals	5 g
Distilled water	100.0 ml

1. Follow the directions listed above for D'Antoni's iodine, including the expiration date of 1 year.
2. Dilute a portion 1:5 with distilled water for routine use (working solution).
3. Place this working solution into a brown dropper bottle. The working solution should have a strong-tea color and should be discarded when the color lightens (usually within 10 to 14 days).

Nair's Buffered Methylene Blue Stain for Trophozoites (Direct Smear)

Although not commonly used, Nair's buffered methylene blue stain is effective in showing nuclear detail in the trophozoite stages when used at a low pH; a pH range of 3.6 to 4.8 allows more active penetration of dye into the organism (15). After 5 to 10 min, the cytoplasm is stained a pale blue, with the nuclei being a darker blue; the slide should be examined within 30 min. Methylene blue (0.06% in an acetate buffer at pH 3.6) usually gives satisfactory results.

Acetate Buffer Solution Stock Solution A (0.2 M)

Acetic acid (CH_3COOH)	11.55 ml
Distilled water	1,000.0 ml

Acetate Buffer Solution Stock Solution B (0.2 M)

Sodium acetate ($NaC_2H_3O_2$)	16.4 g
or	
Sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)	27.2 g
Distilled water	1,000.0 ml

Mix the quantity of stock solutions A and B shown in the following table and dilute with distilled water to a total of 100 ml.

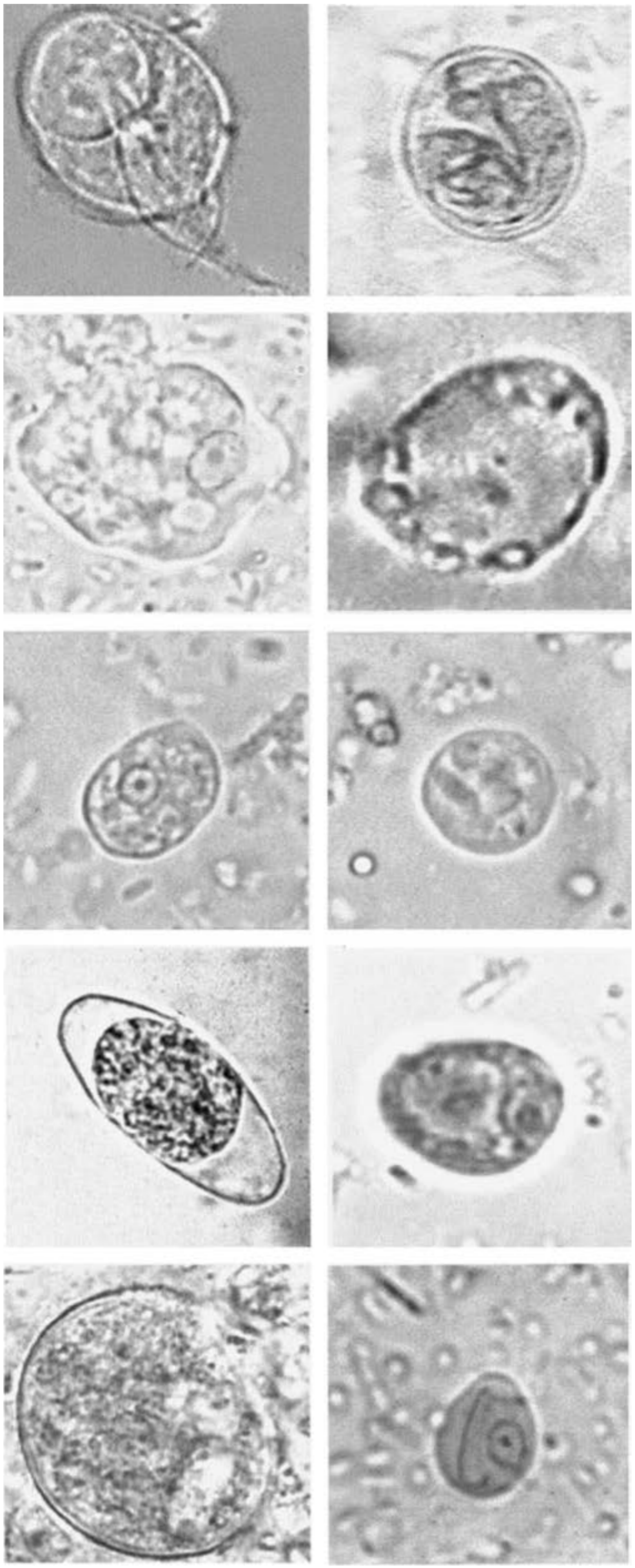


Figure 3.2 Direct wet smear with saline. (Top row) *Giardia lamblia* (*G. duodenalis, G. intestinalis*) trophozoite (left), *G. lamblia* cyst (right); (second row) *Entamoeba* sp. (probably *E. coli*) (left), *Blastocystis* spp. central body form (right); (third row) *Entamoeba hartmanni* trophozoite (left), *E. hartmanni* cyst (right); (fourth row) *Cystoisospora belli* immature oocyst (left), *Iodamoeba bütschlii* cyst (right); (bottom row) *Balantidium coli* cyst (left), *Chilomastix mesnili* cyst (right).
doi:10.1128/9781555819002.ch3.f2

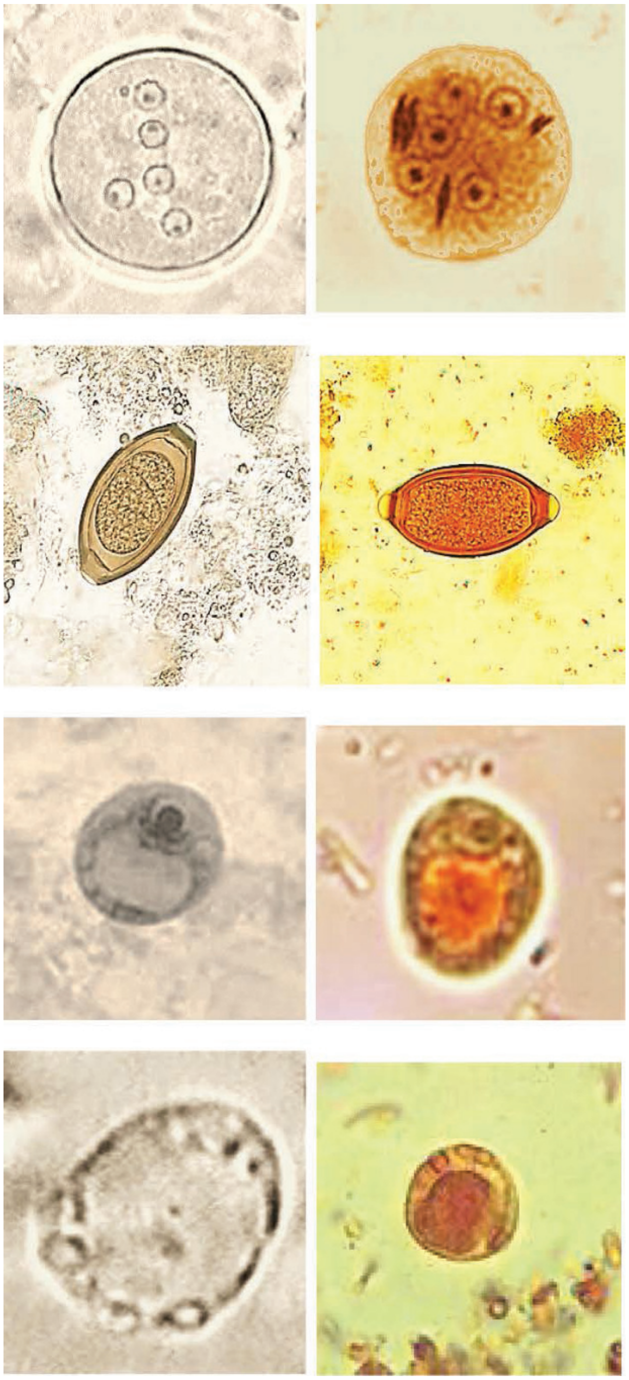


Figure 3.3 Direct wet smear with saline and iodine. (Top) *Entamoeba coli* cyst with saline (left), *E. coli* cyst with iodine (note chromatoidal bars with sharp ends) (right); (next row) *Trichuris trichiura* egg in saline (left), *T. trichiura* egg with iodine added (right); (next row) *Iodamoeba bütschlii* cyst with saline (left), *I. bütschlii* cyst with iodine (right); (bottom row) *Blastocystis* spp. in saline (left), *Blastocystis* spp. in iodine (right). Note that more detail can be seen once the iodine is added to the wet mount. Also, when iodine is used, the glycogen vacuole stains dark (brownish gold to brown) in the *Iodamoeba* cysts and is clearly visible. doi:10.1128/9781555819002.ch3.f3

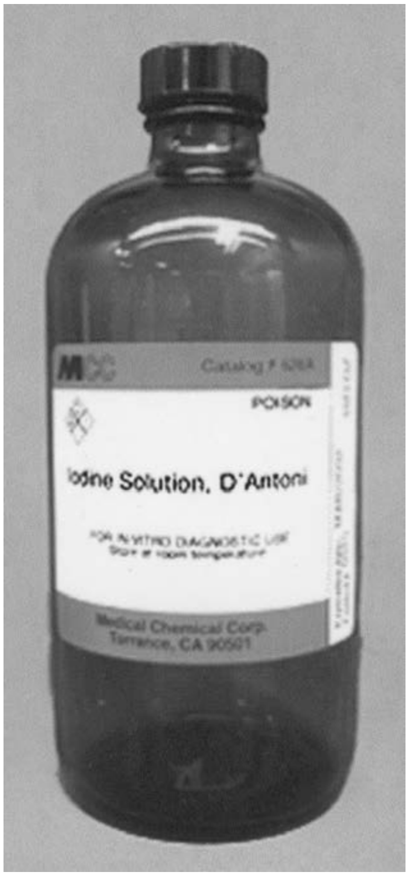


Figure 3.4 Commercially prepared D'Antoni's iodine; most commercial suppliers can provide this iodine solution. Do NOT USE Gram's iodine for the parasitology procedures. doi:10.1128/9781555819002.ch3.f4

Desired pH	Stock solution A (ml)	Stock solution B (ml)
3.6	46.3	3.7
3.8	44.0	6.0
4.0	41.0	9.0
4.2	36.8	13.2
4.4	30.5	19.5
4.6	25.5	24.5

Quality Control for Direct Smear

1. Check the working iodine solution each time it is used or periodically (once a week). The iodine and Nair's methylene blue solutions should be free of any signs of bacterial or fungal contamination.
2. The iodine should be the color of strong tea (discard if it is too light).
3. Protozoan cysts stained with iodine should contain yellow-gold cytoplasm, brown glycogen material, and paler refractile nuclei. The chromatoidal bodies

may not be as clearly visible as they are in a saline mount. Human WBCs (buffy coat cells) mixed with negative stool can be used as a quality control (QC) specimen. These human cells, when mixed with negative stool, mimic protozoan parasites. The human cells stain with the same color as that seen in the protozoa.

4. Protozoan trophozoite cytoplasm should stain pale blue and the nuclei should stain a darker blue with the methylene blue stain. Human WBCs mixed with negative stool should stain the same colors as seen with the protozoa.
5. The microscope should be calibrated (within the last 12 months), and the original optics used for the calibration should be in place on the microscope when objects are measured. Some microbiologists feel that calibration is not required on a yearly basis; however, if the microscope receives heavy use, is in a position where it can be bumped, or does not receive routine maintenance, yearly calibration is recommended. The calibration factors for all objectives should be posted on the microscope or close by for easy access.
6. All QC results should be appropriately recorded; the laboratory should also have an action plan for "out-of-control" results.

Procedure for Direct Wet Smear

1. Place 1 drop of 0.85% NaCl on the left side of the slide and 1 drop of iodine (working solution) on the right side of the slide. If preferred, two slides can be used instead of one. One drop of Nair's methylene blue can also be placed on a separate slide, although this technique is much less common.
2. Take a small amount of fecal specimen (the amount picked up on the end of an applicator stick when introduced into the specimen), and thoroughly emulsify the stool in the saline and iodine preparations (use separate sticks for each).
3. Place a 22-mm coverslip (no. 1) on each suspension.
4. Systematically scan both suspensions with the 10× objective. The entire coverslip area should be examined under low power (total magnification, ×100).
5. If something suspicious is seen, the 40× objective can be used for more detailed study. At least one-third to one-half of the coverslip should be examined under high dry power (total magnification, ×400), even if nothing suspicious has been seen.
6. Another approach is to prepare and examine the saline mount and then add iodine at the side of the coverslip. The iodine will diffuse into the stool-saline mixture, providing some stain for a second examination. Remember, the iodine will kill any organisms present; thus, no motility will be seen after the iodine is added to the preparation. The use of iodine is not mandated, but is based on personal preference.

Results and Patient Reports from Direct Wet Smear

Protozoan trophozoites and/or cysts and helminth eggs and larvae may be seen and identified. In a heavy infection with *Cryptosporidium* spp., *Cyclospora cayetanensis*, or *Cystoisospora* (*Isospora*) *belli*, oocysts may be seen in a direct smear; however, some type of modified acid-fast stain or fecal immunoassay is normally used to detect *Cryptosporidium* spp., particularly when few oocysts are present. *Cyclospora* oocysts are often confirmed using autofluorescence or the modified acid-fast stain. Spores of the microsporidia are too small, and the shape resembles other debris within the stool; therefore, they are not readily visible in a direct smear.

1. Motile trophozoites and protozoan cysts may or may not be identified to the species level (depending on the clarity of the morphology) and should be confirmed using the permanent stained smear.
   Examples: *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) trophozoites
   Entamoeba coli cysts
2. Helminth eggs and/or larvae may be identified.
   Examples: *Ascaris lumbricoides* eggs
   Strongyloides stercoralis larvae
3. *Cystoisospora* (*Isospora*) *belli* oocysts may be identified; however, *Cyclospora* and *Cryptosporidium* oocysts are generally too small to be recognized or identified without subsequent immunoassays or modified acid-fast staining.
   Example: *Cystoisospora* (*Isospora*) *belli* oocysts
4. Artifacts and/or other structures may also be seen and reported as follows.

Note These crystals and cells are quantitated; however, the quantity is usually assessed when the permanent stained smear is examined under oil immersion.

Examples: Moderate Charcot-Leyden crystals
Few RBCs
Moderate PMNs

Procedure Notes for Direct Wet Smear

1. **In preserved specimens, the formalin, sodium acetate-acetic acid-formalin (SAF), or Universal Fixative replaces the saline and can be used as a direct smear; however, no organism motility will be visible (organisms are killed by the fixatives). Consequently, the direct wet smear is usually not performed when the specimen (already preserved)**

arrives in the laboratory. ***The technical time is better spent performing the concentration and permanent stained smear. This approach is recommended for specimens submitted to the laboratory in preservative*** (2).

2. As mentioned above, some workers prefer to make the saline and iodine mounts on separate slides and on 2- by 3-in. slides. Often, there is less chance of getting fluids on the microscope stage if separate slides (less total fluid on the slide and under the coverslip) or larger slides are used. Selection of slide size and the use of iodine depend on the personal preference of laboratory personnel.
3. The microscope light should be reduced for low-power observations, since most organisms are overlooked with bright light due to limited contrast of the internal morphology. This is particularly true when the preparation is being examined without the use of iodine. Illumination should be regulated so that some of the cellular elements in the feces show refraction (lower the condenser). Most protozoan cysts and some coccidian oocysts are refractile under these light conditions.

Procedure Limitations for Direct Wet Smear

1. As mentioned above, because motility is lost when specimens are placed in preservatives, many laboratories are no longer performing the direct wet smear (the primary purpose is to see motility) but are proceeding directly to the concentration and permanent stained smear procedures as a better, more cost-effective use of personnel time, as well as a more clinically relevant approach.
2. Most of the time, results obtained from wet smear examinations should be confirmed by permanent stained smears. Some protozoa are very small and difficult to identify to the species level using just the direct wet smear technique. Confirmation is particularly important for *Entamoeba histolytica*/*E. dispar* versus *Entamoeba coli*. Findings from the direct wet smear examination can be reported as "preliminary, based on the direct wet mount examination only," and the final report can be submitted after the concentration and permanent stain procedures are completed. However, if the laboratory turnaround time is less than 24 h, there is no need to send out a preliminary report; the final report can be submitted once the complete ova and parasite examination has been performed.

Concentration (Sedimentation and Flotation)

Fecal concentration has become a routine procedure as a part of the complete ova and parasite examination for parasites; it allows the detection of small numbers of organisms that may be missed by using only a direct wet smear

REVIEW Direct Smear

Topic	Comments
Principle	To assess worm burden of patient, to provide quick diagnosis of heavily infected specimen, to check organism motility, and to diagnose organisms that might not be seen from concentration or permanent stain methods
Specimen	Any fresh stool specimen that has not been refrigerated (liquid or soft stool)
Reagents	0.85% NaCl; Lugol's or D'Antoni's iodine; Nair's methylene blue can be used for trophozoites (not common). DO NOT USE GRAM'S IODINE.
Examination	Low-power examination (×100) of entire 22- by 22-mm coverslip preparation (both saline and iodine); high dry power examination (×400) of at least one-third of the coverslip area (both saline and iodine). THE USE OF IODINE IS OPTIONAL.
Results and Laboratory Reports	Results from the direct smear examination should often be considered presumptive; however, some organisms could be definitively identified, e.g., *Giardia lamblia* (*G. duodenalis, G. intestinalis*) cysts and *Entamoeba coli* cysts, helminth eggs and larvae, *Cystoisospora* (*Isospora*) *belli* oocysts. These reports should be categorized as "preliminary," while the final report would be available after the results of the concentration and permanent stained smear were available. However, if the testing turnaround time is 24 h, it is best to report the test results after the ova and parasite examination (fresh specimen, direct wet smear; concentration, permanent stained smear) is complete.
Procedure Notes and Limitations	Once iodine is added to the preparation, the organisms are killed and motility is lost. Specimens that arrive in the laboratory already preserved do not require a direct smear examination; the concentration and permanent stained smear should be performed instead. Direct smears are normally examined at low (×100) and high dry (×400) power; oil immersion examination (×1,000) is not recommended (organism morphology not that clear).

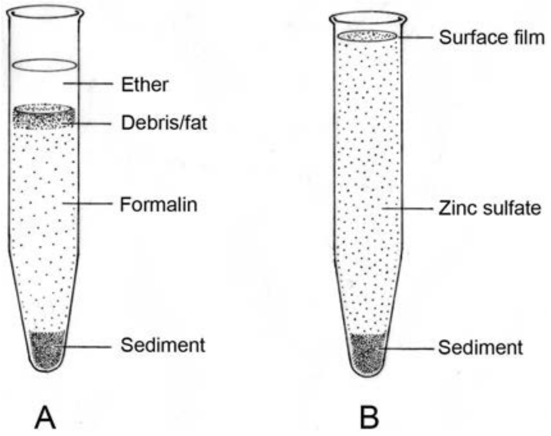


Figure 3.5 Fecal concentration procedures: various layers seen in tubes after centrifugation. (A) Formalin-ether (or ethyl acetate). The sediment should be well mixed, and a drop of sediment should be examined using the 10× low-power objective and the 40× high dry power objective. (B) Zinc sulfate (the surface film should be within 2 to 3 mm of the tube rim). Material from both the surface film and the sediment must be examined before the specimen is indicated as negative. The amount of sediment should not be excessive in either the sedimentation or flotation procedure. Heavy or operculated helminth eggs do not float. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch3.f5

(11, 21). There are two types of concentration procedures, sedimentation and flotation, both of which are designed to separate protozoan organisms and helminth eggs and larvae from fecal debris by centrifugation and/or differences in specific gravity (Fig. 3.5) (2, 4).

Sedimentation methods (by centrifugation) lead to the recovery of all protozoa, oocysts, eggs, and larvae present; however, the concentration sediment that will be examined contains more debris. Although some workers recommend using both flotation and sedimentation procedures for every stool specimen submitted for examination, this approach is impractical for most laboratories. If one technique is selected for routine use, the sedimentation procedure is recommended as being the easiest to perform and the least subject to technical error (Fig. 3.6).

A flotation procedure permits the separation of protozoan cysts, coccidian oocysts, and certain helminth eggs and larvae through the use of a liquid with a high specific gravity. The parasitic elements are recovered in the surface film, and the debris remains in the bottom of the tube. This technique yields a cleaner preparation than does the sedimentation procedure; however, some helminth eggs (operculated eggs and/or very dense eggs such as unfertilized *Ascaris* eggs) do not concentrate well in the flotation method (Fig. 3.7). The specific gravity may be increased, although this may produce more distortion in the eggs and protozoa. Laboratories that use only flotation procedures may fail to recover all of the parasites present; **to ensure detection of all organisms in the sample, both the surface film and the sediment should be carefully examined.** Directions for any flotation technique must be followed exactly to produce reliable results.

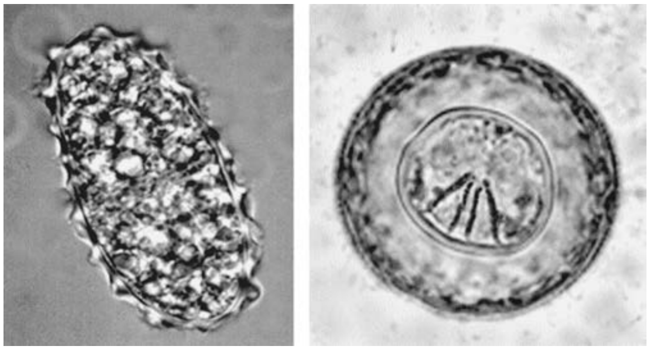


Figure 3.6 Sedimentation concentration. (Left) Unfertilized *Ascaris lumbricoides* egg. (Right) *Hymenolepis diminuta* egg. doi:10.1128/9781555819002.ch3.f6

Formalin-Ethyl Acetate Sedimentation Concentration

By centrifugation, the formalin-ethyl acetate sedimentation concentration procedure leads to the recovery of all protozoa, eggs, larvae, coccidia, and microsporidia present; however, the preparation contains more debris than is found in the flotation procedure. Ethyl acetate is used as an extractor of debris and fat from the feces and leaves the parasites at the bottom of the suspension in the sediment. The formalin-ethyl acetate sedimentation concentration procedure is recommended as being the easiest to perform, allowing recovery of the broadest range of organisms, and being the least subject to technical error.

The specimen must be fresh or formalinized stool (5 or 10% buffered or nonbuffered formalin or SAF or the Universal Fixative). Many of the single-vial preservative systems are also acceptable; however, the formulas are proprietary (e.g., UNIFIX; Medical Chemical Corp., Torrance, CA). Specimens preserved in fixatives containing polyvinyl alcohol (PVA) can also be used. However, PVA preservative formulations are rarely used for concentration methods in most laboratories but are recommended for the preparation of permanent stained smears.

5 or 10% Formalin

Formaldehyde (USP)	
	100 ml (for 10%) or
	50 ml (for 5%)
Saline solution	900 ml (for 10%) or
0.85% NaCl	950 ml (for 5%)

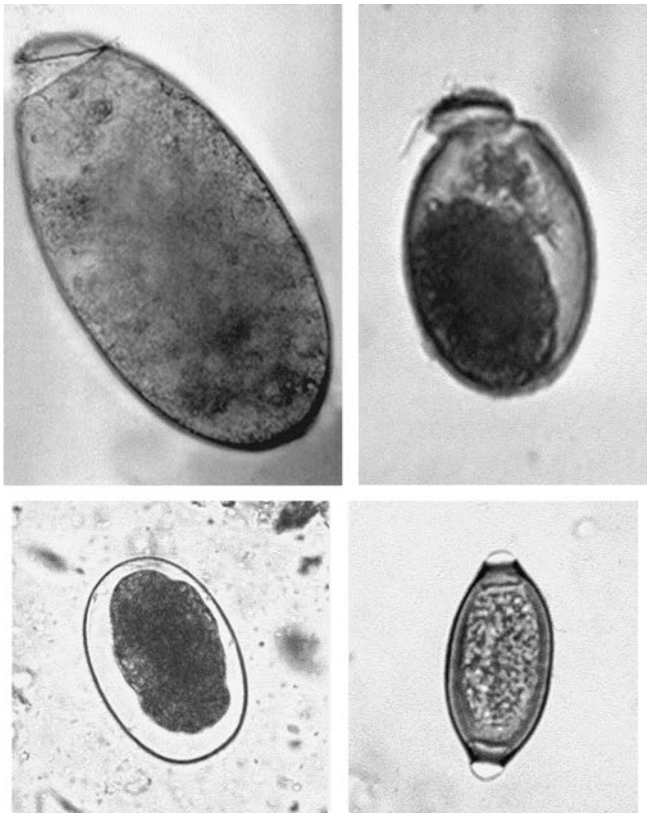


Figure 3.7 Flotation concentration. (Upper) *Fasciolopsis buski* egg (left), *Diphyllobothrium latum* egg (right). Note that both of these eggs in the top row are operculated and WILL NOT float in the zinc sulfate flotation concentration method; the opercula pop open, and the eggs fill with fluid and sink to the bottom of the tube. (Lower) Hookworm egg (left), *Trichuris trichiura* egg (right). These eggs concentrate using the flotation method and can be seen in the surface film. However, remember that both the surface film and the sediment must be examined by this method before reporting the final ova and parasite examination results. doi:10.1128/9781555819002.ch3.f7

Note Formaldehyde is normally purchased as a 37 to 40% HCHO solution; however, for dilution, it should be considered to be 100%.

Dilute 100 ml of formaldehyde with 900 ml of 0.85% NaCl solution. (Distilled water may be used instead of saline solution.)

Quality Control for Sedimentation Concentration

1. Check the liquid reagents each time they are used; the formalin and saline should appear clear, without any visible contamination.
2. The microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be in place on the microscope when objects are measured. The calibration factors for all objectives should be posted on the microscope or close by for easy access. Some researchers feel that a microscope does not require calibration every 12 months; however, if the microscope is moved periodically, can be easily bumped, or does not receive adequate maintenance, it should be rechecked yearly for calibration accuracy.
3. Known positive specimens should be concentrated and organism recovery should be verified at least quarterly and particularly after the centrifuge has been recalibrated. Human WBCs (buffy coat cells) mixed with negative stool can be used as a QC specimen. These human cells, when mixed with negative stool, can mimic protozoan parasites. The human cells concentrate just like human parasites, such as protozoa and helminth eggs and larvae.
4. All QC results should be appropriately recorded; the laboratory should also have an action plan for "out-of-control" results.

Procedure for Sedimentation Concentration

1. Transfer 1/2 teaspoon (about 4 g) of fresh stool into 10 ml of 5 or 10% formalin in a shell vial, unwaxed paper cup, or round-bottom tube (the container may be modified to suit individual laboratory preferences). Mix the stool and formalin thoroughly, and let the mixture stand for a minimum of 30 min for fixation. If the specimen is already in 5 or 10% formalin (or SAF or other non-PVA single-vial preservatives), stir the stool-preservative mixture.
2. Depending on the amount and viscosity of the specimen, strain a sufficient quantity through wet gauze (no more than two layers of gauze or one layer if the new "pressed" gauze [e.g., Johnson & Johnson nonsterile three-ply gauze, product **7636**] is used) into a conical 15-ml centrifuge tube to give the desired amount of sediment (0.5 to 1 ml) for step 3 below. Usually, 8 ml of the stool-formalin mixture prepared in step 1 is sufficient. If the specimen is received in a vial of preservative (5 or 10% formalin, SAF, or other single-vial preservatives), approximately 3 to 4 ml of the preservative-stool mixture is sufficient for testing. If the vial contains very little specimen, then the entire amount may be used in the procedure. *If the specimen contains a lot of mucus, do not strain through gauze but immediately fix in 5 or 10% formalin for 30 min and centrifuge for 10 min at 500 ×* g. *Proceed directly to step 10.*
3. Add 0.85% NaCl or 5 or 10% formalin (some workers prefer to use formalin for all rinses) almost to the top of the tube, and centrifuge for 10 min at

500 × *g*. The amount of sediment obtained should be approximately 0.5 to 1 ml.

4. Decant and discard the supernatant fluid, and resuspend the sediment in saline or formalin; add saline or formalin almost to the top of the tube, and centrifuge again for 10 min at 500 × *g*. This second wash may be eliminated if the supernatant fluid after the first wash is light tan or clear. Some prefer to limit the washing to one step (regardless of the clarity or color of the supernatant fluid after centrifugation) to eliminate additional manipulation of the specimen prior to centrifugation. **The more the specimen is manipulated and/or rinsed, the more likely it is that some organisms will be lost and accidentally discarded prior to examination of the sediment.**
5. Decant and discard the supernatant fluid, and resuspend the sediment on the bottom of the tube in 5 or 10% formalin. Fill the tube half full only. If the amount of sediment left in the bottom of the tube is very small or the original specimen contained a lot of mucus, do not add ethyl acetate in step 6; merely add the formalin, spin, decant, and examine the remaining sediment.
6. Add 4 to 5 ml of ethyl acetate. Stopper the tube, and shake it vigorously for at least 30 s. Hold the tube so that the stopper is directed away from your face.
7. After a 15- to 30-s wait, carefully remove the stopper.
8. Centrifuge for 10 min at 500 × *g*. Four layers should result: a small amount of sediment (containing the parasites) in the bottom of the tube; a layer of formalin; a plug of fecal debris on top of the formalin layer; and a layer of ethyl acetate at the top (Fig. 3.5).
9. Free the plug of debris by ringing the plug with an applicator stick; decant and discard all of the supernatant fluid. After proper decanting, a drop or two of fluid remaining on the side of the tube may run down into the sediment. Mix this fluid with the sediment.
10. If the sediment is still somewhat solid, add 1 or 2 drops of saline or formalin to the sediment, mix, add a small amount of material to a slide, add a coverslip (22 by 22 mm, no. 1), and examine.
11. Systematically scan with the 10× objective. The entire coverslip area should be examined under low power (total magnification, ×100).
12. If something suspicious is seen, the 40× objective can be used for more detailed study. At least one-third to one-half of the coverslip should be examined under high dry power (total magnification, ×400), even if nothing suspicious has been seen. As in the direct wet smear, iodine can be added to enhance morphological detail, and the coverslip can be tapped to see objects move and turn over. The use of iodine is optional.

Results and Patient Reports from Sedimentation Concentration

Protozoan trophozoites and/or cysts and helminth eggs and larvae may be seen and identified. Protozoan trophozoites are less likely to be seen. In a heavy infection with *Cryptosporidium* spp. or *C. cayetanensis*, oocysts may be seen in the concentrate sediment; oocysts of *C. belli* can also be seen. Spores of the microsporidia are too small, and the shape resembles that of other debris within the stool; therefore, they are not readily visible in the concentration sediment. However, special stains can be performed on the sediment for the identification of coccidian oocysts and microsporidian spores.

1. Protozoan cysts may or may not be identified to the species level (depending on the clarity of the morphology).
   Examples: *Entamoeba coli* cysts
   Giardia lamblia (*G. duodenalis, G. intestinalis*) cysts
2. Helminth eggs and/or larvae may be identified.
   Examples: *Ascaris lumbricoides* eggs
   Hookworm larvae
3. *C. belli* oocysts may be identified; however, *Cyclospora* and *Cryptosporidium* oocysts are generally too small to be recognized and can be identified using appropriate immunoassays or modified acid-fast staining.
   Example: *Cystoisospora* (*Isospora*) *belli* oocysts
4. Artifacts and/or other structures may also be seen and reported as follows.

Note These crystals and cells are quantitated; however, the quantity is usually assessed when the permanent stained smear is examined under oil immersion).

Examples: Moderate Charcot-Leyden crystals
Few RBCs
Moderate PMNs

Procedure Notes for Sedimentation Concentration

1. The gauze should never be more than one (pressed gauze) or two (woven gauze) layers thick; more gauze may trap mucus (containing *Cryptosporidium* oocysts and/or microsporidial spores).
2. Tap water may be substituted for 0.85% NaCl throughout this procedure, although the addition of water to fresh stool causes *Blastocystis* spp. cyst (central body) forms to rupture and is not recom-

mended. In addition to the original 5 or 10% formalin fixation, some workers prefer to use 5 or 10% formalin for all rinses throughout the procedure.

3. Ethyl acetate is widely recommended as a substitute for ether (16). It can be used in the same way in the procedure and is much safer. Hemo-De can also be used and is thought to be safer than ethyl acetate (23).
   - A. After the plug of debris is rimmed and excess fluid is decanted, *the sides of the tube can be swabbed with a cotton-tipped applicator stick while the tube is still upside down to remove excess ethyl acetate. This is particularly important if you are working with plastic centrifuge tubes or the plastic commercial concentrators.* If the sediment is too dry after the tube has been swabbed, add several drops of saline before preparing the wet smear for examination.
   - B. If there is excess ethyl acetate in the smear of the sediment prepared for examination, bubbles will be present, which will obscure the material of interest.
4. If specimens are received in SAF, begin the procedure at step 2.
5. *If specimens are received in fixative containing PVA, the first two steps of the procedure should be modified as follows.*
   - A. Immediately after stirring the stool-PVA mixture with applicator sticks, pour approximately half of the mixture into a tube (container optional) and add 0.85% NaCl (or 5 or 10% formalin) almost to the top of the tube.
   - B. Filter the stool-PVA-saline (or formalin) mixture through wet gauze into a 15-ml centrifuge tube. Follow the standard procedure from here to completion, beginning with step 3.
6. Too much or too little sediment will result in an ineffective concentration sediment examination. **See the section on Commercial Fecal Concentration Devices later in this chapter.**
7. The centrifuge should reach the recommended speed before the centrifugation time is monitored. *However, since most laboratories have their centrifuges on automatic timers, the centrifugation time in this protocol takes into account the fact that some time will be spent coming up to speed prior to full-speed centrifugation.* ***If the centrifugation time at the proper speed (10 min at 500 × g) is reduced, some of the organisms (*****Cryptosporidium*** ***and*** **Cyclospora** ***oocysts or microsporidian spores) may not be recovered in the sediment.***

Procedure Limitations for Sedimentation Concentration

1. Results obtained with wet smears (direct wet smears or concentration sediment wet smears) should usually be confirmed by permanent stained smears. Some protozoa are very small and difficult to identify as to species with just the direct wet smears. Also, special stains are sometimes necessary for organism identification.
2. Confirmation is particularly important for *E. histolytica*/*E. dispar* versus *E. coli*.
3. Certain organisms [*G. lamblia* (*G. duodenalis, G. intestinalis*), hookworm eggs, and occasionally *Trichuris* eggs] may not concentrate as well from PVA-preserved specimens as they do from those preserved in formalin. However, if enough *Giardia* organisms are present to concentrate from formalin, PVA should contain enough for detection on the permanent stained smear. In clinically important infections, the number of helminth eggs present would ensure detection regardless of the type of preservative used. Also, the morphology of *Strongyloides stercoralis* larvae is not as clear from specimens in PVA as from specimens fixed in formalin.
4. For unknown reasons, *C. belli* oocysts are routinely missed in the concentrate sediment when concentrated from PVA-preserved specimens. The oocysts would be found if the same specimen were preserved in formalin rather than PVA.
5. In past publications, recommended centrifugation times have not taken into account potential problems with the recovery of *Cryptosporidium* oocysts. **There is anecdotal evidence strongly indicating that *Cryptosporidium* oocysts may be missed unless the centrifugation speed is 500 × *g* for a minimum of 10 min.**
6. Adequate centrifugation time and speed have become very important for recovery of microsporidial spores. In some of the earlier publications, use of uncentrifuged material was recommended. **However, we have found that centrifugation for 10 min at 500 × g definitely increases the number of microsporidial spores available for staining and subsequent examination.**

Iodine-Trichrome Stain for Sediment

A combination of Lugol's iodine solution and trichrome stain can be used to stain fecal sediment from the concentration procedure (24). Coloring the eggs and cysts yellow-brown (iodine) and the debris green (trichrome) provides contrast which facilitates the detection of parasites. The use of such an approach usually depends on personal preferences and the results of parallel trials of the current method and new methods being considered. This iodine-trichrome wet examination can be used as an adjunct procedure *but does not take the place of the unstained wet examination of the sediment*.

Quality Control for Iodine-Trichrome Stain for Sediment

1. Check the working iodine solution each time it is used or periodically (once a week). The iodine and trichrome stain solutions should be free of any signs of bacterial or fungal contamination.
2. The iodine should be the color of strong tea (discard if it is too light).
3. Protozoan cysts stained with iodine should contain yellow-gold cytoplasm, brown glycogen material, and paler refractile nuclei. The chromatoidal bodies may not be as clearly visible as in a saline mount. Human WBCs (buffy coat cells) mixed with negative stool can be used as a QC specimen. The human cells stain with the same color as that seen in the protozoa. The background debris stains green from the components of the trichrome stain.
4. If appropriate due to extensive use and/or lack of routine maintenance, the microscope should be calibrated (within the last 12 months), and the original optics used for the calibration should be in place on the microscope when objects are being measured. The calibration factors for all objectives should be posted on the microscope or close by for easy access.
5. All QC results should be appropriately recorded; the laboratory should also have an action plan for "out-of-control" results.

Procedure for Iodine-Trichrome Stain for Sediment

1. Place 4 drops of Lugol's iodine solution into a test tube.
2. Place 4 drops of fecal concentrate into the test tube. Mix well.
3. Place 2 drops of the Lugol's iodine solution-fecal concentrate mixture from step 2 on a glass slide.
4. Add 1 drop of trichrome stain. Mix with a wooden applicator stick, and cover with a coverslip (22 by 22 mm, no. 1).
5. Microscopically examine the entire preparation under low power (×100) and at least one-third to one-half of the area under high dry power (×400).

Results and Patient Reports from Iodine-Trichrome Stain for Sediment

Protozoan trophozoites and/or cysts and some helminth eggs and larvae may be seen and identified. Lugol's iodine stains *A. lumbricoides* and *Taenia* eggs quite dark; these eggs are difficult to recognize and may be mistaken for debris, hence the need for the unstained sediment examination. In a heavy infection with *Cryptosporidium* spp., oocysts may be seen in a direct smear; however, some type of modified acid-fast stain or fecal immunoassay is normally used to detect these organisms, particularly when few oocysts are present. Oocysts of *C. belli* can also be seen in a direct smear. *Cyclospora* oocysts may not be recognized because they resemble debris. Spores of the microsporidia are too small, and the shape resembles that of other debris within the stool; therefore, they are not readily visible in a direct smear.

1. Protozoan cysts may or may not be identified to the species level (depending on the clarity of the morphology).
   Example: *Iodamoeba bütschlii* cysts
2. Helminth eggs and/or larvae may be identified.
   Example: *Hymenolepis nana* eggs
3. *C. belli* oocysts may be identified; however, *Cyclospora* and *Cryptosporidium* oocysts are generally too small to be recognized or identified. Subsequent immunoassays or modified acid-fast staining is recommended.
   Example: *Cystoisospora* (*Isospora*) *belli* oocysts
4. Artifacts and/or other structures may also be seen and reported as follows.

Note These cells are quantitated; however, the quantity is usually assessed when the permanent stained smear is examined under oil immersion.

Example: Moderate PMNs

Procedure Notes for Iodine-Trichrome Stain for Sediment

1. As mentioned above, some workers prefer to make the wet mounts on larger slides. Often, there is less chance of getting fluids on the microscope stage if larger slides are used.
2. This stain is darker than the traditional iodine stain. The microscope light should be increased over that used for the unstained wet smear examination.

Procedure Limitations for Iodine-Trichrome Stain for Sediment

1. Because this is a darker stain than routine iodine stains, it is important to also examine a saline wet smear. This is particularly important because *A. lumbricoides* and *Taenia* eggs stain too dark with

the iodine and may not be recognized as helminth eggs.

2. Results obtained with wet smears should usually be confirmed by permanent stained smears. Some protozoa are very small and difficult to identify to the species level with just the direct wet smears. Confirmation is particularly important for *E. histolytica*/*E. dispar* versus *E. coli*. These findings can be reported as "preliminary report, based on direct wet smear examination only," and the final report can be submitted after the concentration and permanent stain procedures are completed. However, if the examination turnaround time is approximately 24 h or less, there is no need for a preliminary report; the final report can be submitted after completion of the concentration and permanent stained smear examinations.

Zinc Sulfate Flotation Concentration

The flotation procedure permits the separation of protozoan cysts and eggs of certain helminths from excess debris through the use of a liquid (zinc sulfate) with a high specific gravity. The parasitic elements are recovered in the surface film, and the debris and some heavy parasitic elements remain in the bottom of the tube. This technique yields a cleaner preparation than does the sedimentation procedure; however, some helminth eggs (operculated and/or very dense eggs, such as unfertilized *Ascaris* eggs) do not concentrate well in the flotation method; a sedimentation technique is recommended to detect these infections.

When the zinc sulfate solution is prepared, the specific gravity should be 1.18 for fresh stool specimens; it must be checked with a hydrometer. This procedure may be used on formalin-preserved specimens if the specific gravity of the zinc sulfate is increased to 1.20; however, this usually causes more distortion in the organisms present and is not recommended for routine clinical use. **To ensure detection of all possible organisms, both the surface film and the sediment must be examined.** For most laboratories, this is not a practical approach.

The specimen must be fresh or formalinized stool (5 or 10% buffered or nonbuffered formalin, SAF, or other non-PVA single-vial preservatives). PVA-preserved specimens can also be used; however, this approach is not commonly used or recommended.

Zinc Sulfate (33% Aqueous Solution)

Zinc sulfate	330 g
Distilled water	670 ml

1. Using a magnetic stirrer, dissolve the zinc sulfate in distilled water in an appropriate flask or beaker.
2. Adjust the specific gravity to 1.20 by the addition of more zinc sulfate or distilled water. Use a specific gravity of 1.18 with fresh stool (nonformalinized).
3. Store in a glass-stoppered bottle with an expiration date of 24 months.

Quality Control for Flotation Concentration

1. Check the reagents each time they are used. The formalin, saline, and zinc sulfate should appear clear, without any visible contamination.
2. The microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope or close by for easy access. As mentioned above, some workers feel that recalibration of the microscope is not necessary each year; however, this would depend on the use and maintenance of that particular piece of equipment.
3. Known positive specimens should be concentrated and organism recovery should be verified at least quarterly, particularly after the centrifuge has been recalibrated. Human WBCs (buffy coat cells) mixed with negative stool can be used as a QC specimen. These human cells, when mixed with negative stool, mimic human parasites. The human cells concentrate just like human parasites, such as protozoa and helminth eggs and larvae.
4. All QC results should be appropriately recorded; the laboratory should also have an action plan for "out-of-control" results.

Procedure for Flotation Concentration

1. Transfer 1/2 teaspoon (about 4 g) of fresh stool into 10 ml of 5 or 10% formalin in a shell vial, unwaxed paper cup, or round-bottom tube (the container may be modified to suit individual laboratory preferences). Mix the stool and formalin thoroughly. Let the mixture stand for a minimum of 30 min for fixation. If the specimen is already in 5 or 10% formalin (or SAF or Universal Fixative), stir the stool-formalin (or SAF) mixture.
2. Depending on the size and density of the specimen, strain a sufficient quantity through wet gauze (no more than two layers of gauze or one layer if the new "pressed" gauze [e.g., Johnson & Johnson nonsterile three-ply gauze, product **7636**] is used) into a conical 15-ml centrifuge tube to give the desired amount of sediment (0.5 to 1 ml) in step 3 below. Usually, 8 ml of the stool-formalin mixture prepared in step 1 is sufficient. If the specimen is received in vials of preservative (5 or 10% formalin, SAF, or other single-vial preservatives), approximately 3 to 4 ml of the mixture is sufficient unless the specimen has very little stool in the vial. *If the*

specimen contains a lot of mucus, do not strain through gauze but immediately fix in 5 or 10% formalin or other fixative (does not contain PVA) for 30 min and centrifuge for 10 min at 500 × g*. Proceed directly to step 5.*

3. Add 0.85% NaCl almost to the top of the tube, and centrifuge for 10 min at 500 × *g*. Approximately 0.5 to 1 ml of sediment should be obtained. Too much or too little sediment results in an ineffective concentration examination. **See the section on Commercial Fecal Concentration Devices later in this chapter.**
4. Decant and discard the supernatant fluid, resuspend the sediment in 0.85% NaCl almost to the top of the tube, and centrifuge for 10 min at 500 × *g*. This second wash may be eliminated if the supernatant fluid after the first wash is light tan or clear. Some prefer to limit the washing to one step (regardless of the color and clarity of the supernatant fluid) to eliminate additional manipulation of the specimen prior to centrifugation. The more the specimen is manipulated and/or rinsed, the more likely it is for parasitic elements to be lost.
5. Decant and discard the supernatant fluid, and resuspend the sediment on the bottom of the tube in 1 to 2 ml of zinc sulfate. Fill the tube within 2 to 3 mm of the rim with additional zinc sulfate.
6. Centrifuge for 2 min at 500 × *g*. Allow the centrifuge to come to a stop without interference or vibration. Two layers should result: a small amount of sediment in the bottom of the tube, and a layer of zinc sulfate (Fig. 3.5). The protozoan cysts and some helminth eggs are found in the surface film; some operculated and/or heavy eggs are found in the sediment.
7. Without removing the tube from the centrifuge, remove 1 or 2 drops of the surface film with a Pasteur pipette or a freshly flamed (and allowed to cool) wire loop and place them on a slide. Do not use the loop as a "dipper"; simply touch the surface (bend the loop portion of the wire 90° so that the loop is parallel with the surface of the fluid). Make sure the pipette tip or wire loop is not below the surface film (Fig. 3.8).
8. Add a coverslip (22 by 22 mm, no. 1) to the preparation. Iodine may be added to the preparation (optional).
9. Systematically scan with the 10× objective. The entire coverslip area should be examined under low power (total magnification, ×100).
10. If something suspicious is seen, the 40× objective can be used for more detailed study. At least one-third to one-half of the coverslip should be examined with high dry power (total magnification, ×400), even if nothing suspicious has been seen. As in the direct wet smear, iodine can be added to enhance morphological detail, and the coverslip can be gently tapped to observe objects moving and turning over.

Results and Patient Reports from Flotation Concentration

Protozoan trophozoites and/or cysts and some helminth eggs and larvae may be seen and identified. Heavy helminth eggs and operculated eggs do not float in zinc sulfate; they are seen in the sediment within the tube. The high specific gravity of the zinc sulfate causes the opercula to pop open; the eggs fill with fluid and sink to the bottom. Protozoan trophozoites are less likely to be seen. In a heavy infection with *Cryptosporidium* spp. or *C. cayetanensis*, oocysts may be seen in the concentrate sediment; oocysts of *C. belli* can also be seen. Spores of the microsporidia are too small, and the shape resembles other debris within the stool; therefore, they are not readily visible in the concentration sediment.

1. Protozoan cysts may or may not be identified to the species level (depending on the clarity of the morphology).
   Example: *Giardia lamblia* (*G. duodenum, G. intestinalis*) cysts
2. Helminth eggs and/or larvae may be identified.
   Example: Hookworm eggs
3. *C. belli* oocysts may be identified; however, *Cyclospora* and *Cryptosporidium* oocysts are generally too small to be recognized or identified. Subsequent immunoassays or modified acid-fast staining is recommended.
   Example: *Cystoisospora* (*Isospora*) *belli* oocysts
4. Artifacts and/or other structures may also be seen and reported as follows.
   Note These cells are quantitated; however, the quantity is usually assessed when the permanent stained smear is examined.
   Examples: Few macrophages
   Moderate PMNs

Procedure Notes for Flotation Concentration

1. The gauze should never be more than one or two layers thick; more gauze may trap mucus (containing *Cryptosporidium* oocysts, *Cyclospora* oocysts, and/or microsporidial spores). A round-bottom tube is recommended rather than a centrifuge tube.
2. Tap water may be substituted for 0.85% NaCl throughout this procedure, although the addition of water to fresh stool causes *Blastocystis* spp.

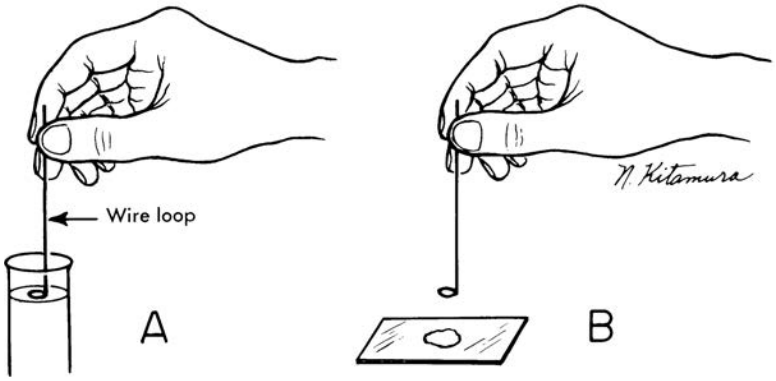


Figure 3.8 Method used to remove the surface film in the zinc sulfate flotation concentration procedure. (A) A wire loop is gently placed on (not under) the surface film. (B) The loop is then placed on a glass slide. (Illustration by Nobuko Kitamura.)
doi:10.1128/9781555819002.ch3.f8

(*Blastocystis hominis*) cyst (central body) forms to rupture and is not recommended. In addition to the original 5 or 10% formalin fixation, some workers prefer to use 5 or 10% formalin for all rinses throughout the procedure.

3. If fresh stool is used (nonformalin preservatives), the zinc sulfate should be prepared with a specific gravity of 1.18. If formalinized specimens are to be concentrated, the zinc sulfate should have a specific gravity of 1.20.
4. If specimens are received in SAF or other single-vial preservatives, begin the procedure at step 2.
5. If fresh specimens are received, the standard procedure requires the stool to be rinsed in distilled water prior to the addition of zinc sulfate in step 4. *However, the addition of fresh stool to distilled water will destroy any* Blastocystis *spp.* (Blastocystis hominis) *cysts present and is not a recommended approach.*
6. Some workers prefer to remove the tubes from the centrifuge prior to sampling the surface film. This is acceptable; however, there is more chance that the surface film will be disturbed prior to sampling.
7. Some workers prefer to add a small amount of zinc sulfate to the tube so that the fluid forms a slightly convex meniscus. A coverslip is then placed on top of the tube so that the undersurface touches the meniscus. It is left undisturbed for 5 min. The coverslip is then carefully removed and placed on a slide for examination. This approach tends to be somewhat messy, particularly if too much zinc sulfate has been added.
8. When using the hydrometer (solution at room temperature), mix the solution well. Float the hydrometer in the solution, giving it a slight twist to ensure that it is completely free from the sides of the container. Read the bottom meniscus and correct the figure for temperature, if necessary. Most hydrometers are calibrated at 20°C. A difference of 3°C between the solution temperature (room temperature) and the hydrometer calibration temperature requires a correction of 0.001, to be added if above and subtracted if below 20°C.

Procedure Limitations for Flotation Concentration

1. Results obtained with wet smears (direct wet smears or concentrated specimen wet smears) should usually be confirmed by permanent stained smears. Some protozoa are very small and difficult to identify to the species level with just the direct wet smears. Also, special stains are sometimes necessary for organism identification.
2. Confirmation is particularly important for *E. histolytica/E. dispar* versus *E. coli*.
3. Protozoan cysts and thin-shelled helminth eggs are subject to collapse and distortion when left for more than a few minutes in contact with the high-specific-gravity zinc sulfate. The surface film should be removed for examination within 5 min of the time the centrifuge comes to a stop. The longer the organisms are in contact with the zinc sulfate, the more distortion will be seen on microscopic examination of the surface film.
4. *Since most laboratories have their centrifuges on automatic timers, the centrifugation time in this protocol takes into account the fact that some time will be spent coming up to speed prior to full-speed centrifugation.*
5. **If zinc sulfate is the only concentration method used, both the surface film and the sediment must be examined to ensure detection of all possible organisms.**

Commercial Fecal Concentration Devices

There are a number of commercially available fecal concentration devices which may help a laboratory to standardize the concentration technique. Standardization is particularly important when personnel rotate throughout the laboratory and may not be familiar with parasitology techniques. These devices help ensure consistency, thus leading

REVIEW Concentration	
Topic	**Comments**
Principle	To concentrate the parasites present, either through sedimentation or by flotation. The concentration is specifically designed to allow recovery of protozoan cysts, coccidian oocysts, microsporidian spores, and helminth eggs and larvae.
Specimen	Any stool specimen that is fresh or preserved in formalin, fixatives containing PVA (mercury- or non-mercury based), SAF, MIF, the single-vial-system fixatives, or Universal Fixative. *Fixatives that contain PVA can be used if no other specimen is available, but are rarely used for fecal concentrations.*
Reagents	5% or 10% formalin, ethyl acetate, zinc sulfate (specific gravity, 1.18 for fresh stool and 1.20 for preserved stool); 0.85% NaCl; Lugol's or D'Antoni's iodine
Examination	Low-power examination (×100) of entire 22- by 22-mm coverslip preparation (iodine recommended but optional); high dry power examination (×400) of at least one-third to one-half of the coverslip area (both saline and iodine)
Results and Laboratory Reports	Often, results from the concentration examination should be considered presumptive; however, some organisms could be definitively identified, e.g., *Giardia lamblia* (*G. duodenalis, G. intestinalis*) cysts and *Entamoeba coli* cysts, helminth eggs and larvae, *Cystoisospora* (*Isospora) belli* oocysts. These reports should be categorized as "preliminary," while the final report would be available after the results of the concentration and permanent stained smear were available. However, if the testing turnaround time is 24 h, it is best to report the test results after the ova and parasite examination (fresh specimen, direct wet smear; concentration, permanent stained smear) is complete.
Procedure Notes and Limitations	Formalin-ethyl acetate sedimentation concentration is the most commonly used. Zinc sulfate flotation does not detect operculated or heavy eggs (will sink to the bottom of the tube); both the surface film and sediment must be examined before a negative result is reported. Smears prepared from concentrated stool are normally examined at low (×100) and high dry (×400) power; oil immersion examination (×1,000) is not recommended (organism morphology is not that clear). The addition of too much iodine may obscure helminth eggs (will mimic debris). Remember that the use of iodine is optional.

to improved parasite recovery and subsequent identification. Some of the systems are enclosed and provide a clean, odor-free approach to stool processing, features that may be important to nonmicrobiology personnel processing such specimens. Both 15- and 50-ml systems are available. **It is important to remember that a maximum of 0.5 to 1.0 ml of sediment is needed in the bottom of the tube. Often, when the 50-ml systems are used, there is too much sediment in the bottom of the tube. This problem can be solved by adding less of the fecal specimen to the concentration system prior to centrifugation. Since the sediment is normally mixed thoroughly and 1 drop is taken to a coverslip for examination, good mixing may not occur if too much sediment is used.** There also appears to be layering in the bottom of the tubes; again, adding less material to the concentrator at the beginning should help eliminate this problem (Fig. 3.9 through 3.12).

Automated Workstation for the Microscopic Analysis of Fecal Concentrates

The FE-5 (Apacor Ltd USA, Brooklyn, NY) is a countertop workstation that automates the microscopic analysis of fecal concentrates (Fig. 3.13). The system automates the aspiration, resuspension, staining or diluting (based on user preference), transfer, presentation, and disposal of fecal concentrates. When the sample button is pressed, within 5 s two samples of fecal concentrate are automatically and simultaneously aspirated from the concentrate tube and transported to the glass dual-flow-cells of the Optical Slide Assembly (Fig. 3.14). Based on user preference, the FE-5 also simultaneously stains or dilutes one of the two samples to be examined. After the microscopic examination of the fecal suspension within the glass viewing chambers, the flow chambers can be purged and cleaned and are ready for the next specimen. The dual-flow-cell Optical Slide Assembly is designed to fit within the stage clips of any standard upright microscope. The Optical Slide Assembly accommodates bright-field, phase-contrast, polarized-light, and other common forms of microscopy. The system can be moved from one microscope to another or can be set up as a semipermanent station for fecal concentrate microscopy. Removal to another microscope just involves removing the Optical Slide Assembly from the microscope stage.

Permanent Stained Smear

The detection and correct identification of many intestinal protozoa frequently depend on the examination of the permanent stained smear with the oil immersion lens (100×

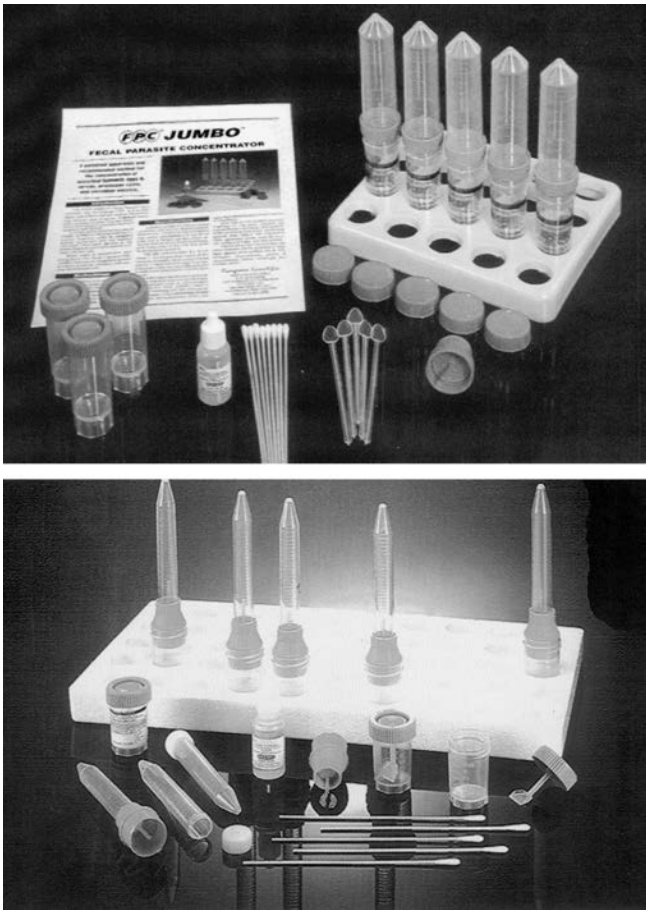


Figure 3.9 (Upper) FPC JUMBO large concentration tubes and connector system (Evergreen Scientific). (Lower) Small concentration tubes and FPC HYBRID connector system (Evergreen Scientific). doi:10.1128/9781555819002.ch3.f9

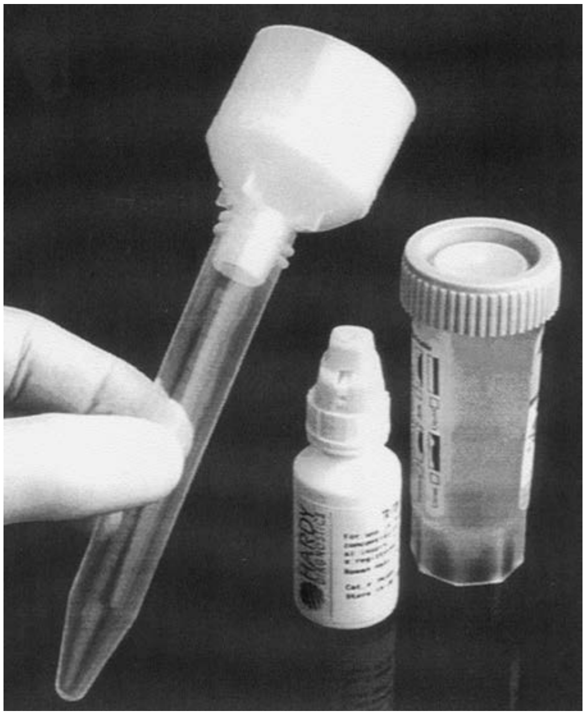


Figure 3.10 Stool collection vial and funnel used in fecal concentration (Hardy Diagnostics). doi:10.1128/9781555819002.ch3.f10

objective). These slides not only provide the microscopist with a permanent record of protozoan organisms identified but also may be used for consultations with specialists when unusual morphologic characteristics are found. Considering the morphologic variations that are possible, organisms that are very difficult to identify and do not fit the pattern for any one species may be found. A routine work flow diagram including the permanent stained smear is shown in Fig. 3.15.

Although an experienced microscopist can occasionally identify certain organisms on a wet preparation, most identifications should be considered tentative until confirmed by the permanent stained slide. The smaller protozoan organisms are frequently seen on the stained smear when they are easily missed with only the direct smear and concentration methods. *For these reasons, the permanent stain is recommended for every stool sample submitted for a routine parasite examination*. It is also important to remember that the fecal immunoassays for specific organisms have proven to be more sensitive than the routine or specialized stains for *Giardia lamblia* (*G. duodenalis, G. intestinalis*) or *Cryptosporidium* spp. (25, 26).

There are a number of staining techniques available; selection of a particular method may depend on the degree of difficulty of the procedure and the amount of time necessary to complete the stain. The older classical method is the long Heidenhain iron hematoxylin method; however, for routine diagnostic work, most laboratories select one of the shorter procedures, such as the trichrome method or one of the modified methods involving iron hematoxylin. Other procedures are available (4, 6–8, 13, 17, 20, 24, 27, 30, 49), and some of them are presented here.

Most problems encountered in the staining of protozoan trophozoites and cysts in fecal smears occur because the specimen is too old, the smears are too dense, the smears are allowed to dry before fixation, or fixation is inadequate. There is variability in fixation in that immature cysts fix more easily than mature cysts, and *E. coli* cysts require a longer fixation time than do those of other species. It is critical that adequate mixing occur between the fecal specimen and preservative (27–29, 31, 32).

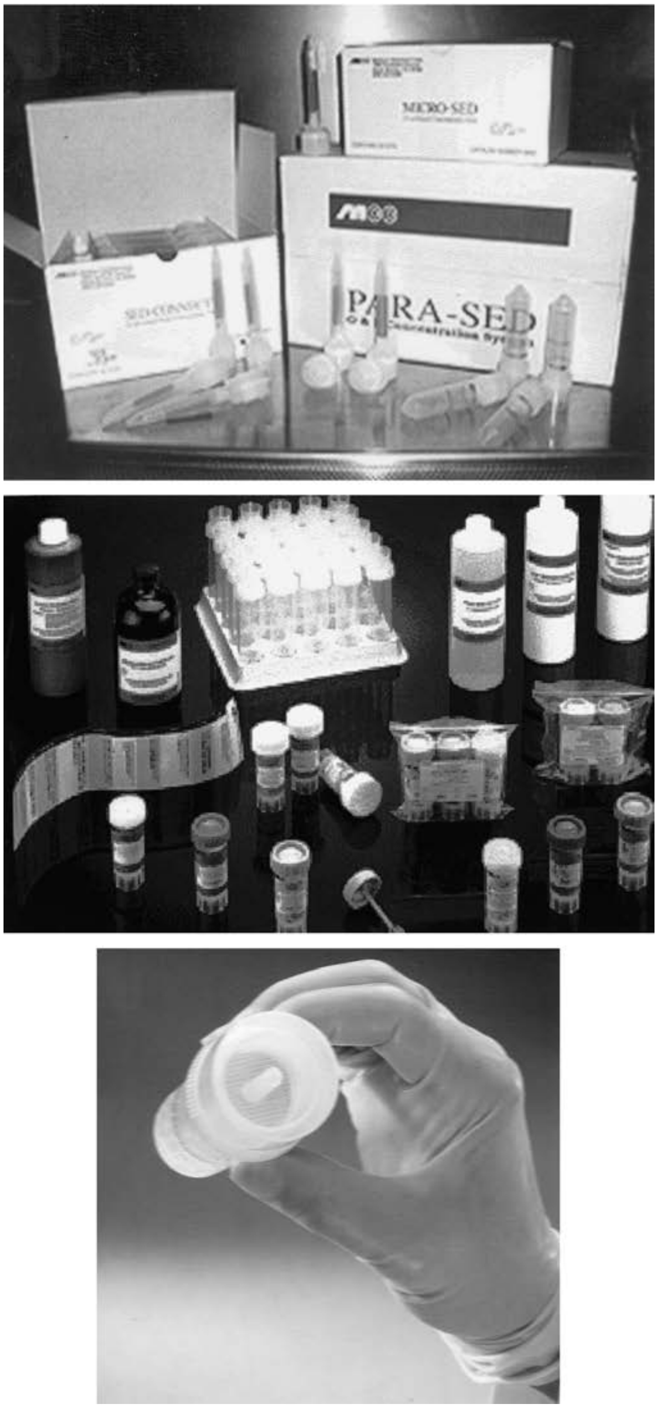


Figure 3.11 (Top) PARA-SED concentration system with both small and large tubes (Medical Chemical Corp.). (Middle) SED-CONNECT system with collection vials and various reagents (Medical Chemical Corp.). (Bottom) Filter attachment system (Medical Chemical Corp.). Note the screen, which is a substitute for the gauze in the traditional gauze/filter concentration method. See also MICRO-SED. doi:10.1128/9781555819002.ch3.f11

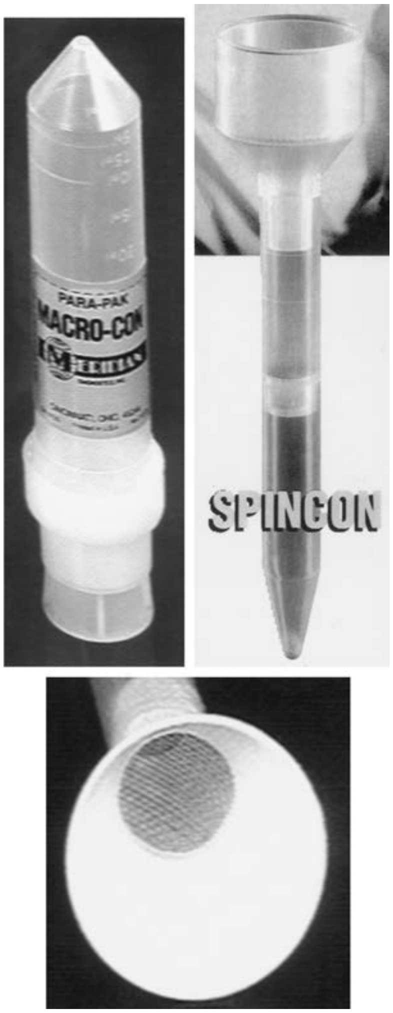


Figure 3.12 (Upper left) MACRO-CON concentration system (Meridian Bioscience). (Upper right) SPINCON concentration system (Meridian Bioscience). (Lower) Funnel used in fecal concentration (Meridian Bioscience). doi:10.1128/9781555819002.ch3.f12

Preparation of Material for Staining

Fresh Material

1. When the specimen arrives, prepare two slides with applicator sticks or brushes and *immediately* (without drying) place them in Schaudinn's fixative [(This approach is rarely used because the formulation contains mercury). Preserved stool collected in fecal fixatives (with or without PVA) can be smeared on a slide and allowed to air dry prior to staining.] Allow the slides to fix for a minimum of 30 min; overnight fixation is acceptable. The amount of fecal material smeared on the slide should be thin enough that newsprint can be read through the smear. Smears preserved in liquid Schaudinn's fixative should be placed in 70% alco-

hol to remove the excess fixative prior to placement in iodine-alcohol (used for mercury-based fixatives).

2. If the fresh specimen is liquid, place 3 or 4 drops of fixative (Schaudinn's fixative to which has been added a plastic powder [PVA], which serves as an adhesive to "glue" the fecal material onto the slide) on the slide, mix several drops of fecal material with the PVA fixative, spread the mixture, and allow it to dry for several hours in a 37°C incubator or overnight at room temperature.
3. Proceed with the trichrome staining procedure by placing the slides in iodine-alcohol.

Preserved Material Containing PVA

1. Stool specimens that are preserved in fixative (mercuric chloride, copper sulfate, or zinc sulfate bases) containing PVA should be allowed to fix for at least 30 min. Thoroughly mix the contents of the vial or bottle (fixative/stool/PVA specimen) with two applicator sticks.
2. Pour some of the well-mixed fixative/stool/PVA mixture onto a paper towel, and allow it to stand for 3 min to absorb out the excess PVA. *Do not eliminate this step.*
3. With an applicator stick (or brush), apply some of the stool material from the paper towel to two slides and allow them to dry for several hours in a 37°C incubator or overnight at room temperature.

   Note The fixative/stool/PVA mixture should be spread to the edges of the glass slide; this will cause the film to adhere to the slide during staining. It is also important to thoroughly dry the slides in order to prevent the material from washing off during staining.
4. The dry slides may then be placed into iodine-alcohol. There is no need to give them a 70% alcohol rinse before placing them in the iodine-alcohol, because the PVA smears are already dry (unlike the wet smears coming out of Schaudinn's fixative). The iodine-alcohol is used to remove the mercuric chloride from the slides before they are stained. When reviewing the trichrome staining procedure, you will note that this step is not required if the stool specimen is preserved in a copper sulfate or zinc sulfate-based preservative.

NOTE Polyvinyl alcohol (PVA) is a plastic powder that is often added to fecal fixatives to help "glue" the stool material onto the glass slide. PVA is inert and has no fixing properties. Its main purpose is to cause the fecal material to adhere to the glass once the material is dry. HOWEVER, the Universal Fixatives do not require PVA or albumin to serve as adhesives.

California State Department of Health Modification (1) for PVA-Preserved Material

1. Thoroughly mix the fixative/stool/PVA mixture, and strain it through damp (with tap water) gauze into 15-ml centrifuge tubes.
2. After centrifugation (2 min at 500 × *g*), decant and discard the fluid and swab the sides of the tubes to remove any excess liquid. Although stains for *Cryptosporidium* spp. are not recommended from preserved material containing PVA, **the centrifuge speed indicated here is probably not sufficient to recover the oocysts or microsporidian spores for special staining, fecal immunoassays using fluorescence, or other diagnostic procedures; use 500 × *g* for 10 min.**
3. Use the remaining sediment to prepare **coverslip smears** (not 3- by 1-in. slides), which are immediately (without drying) stained, beginning with the iodine-alcohol step of the trichrome staining procedure (for removal of mercuric chloride).
4. The staining times can be reduced to 2 to 4 min, and dehydration steps can be reduced to 1 min each.

Note This procedure will work quite well for most stool specimens preserved in fixative containing PVA. However, for very liquid specimens or those containing large amounts of mucus, drying the smears before staining will be advantageous.

SAF-Preserved Material (31)

1. Thoroughly mix the SAF-stool mixture, and strain it through gauze into a 15-ml centrifuge tube.

Figure 3.13 Countertop workstation that automates the microscopic analysis of fecal concentrates (Apacor Ltd. USA, Brooklyn, NY). doi:10.1128/9781555819002.ch3.f13

APACOR
FE-5
Cycle Time
Diagnostics
Unstained
Stained
Under Pressured
Over Pressured
System Error
Power
Sample
Purge
Unstained
Stained

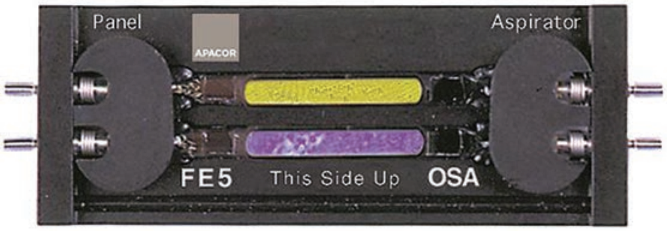
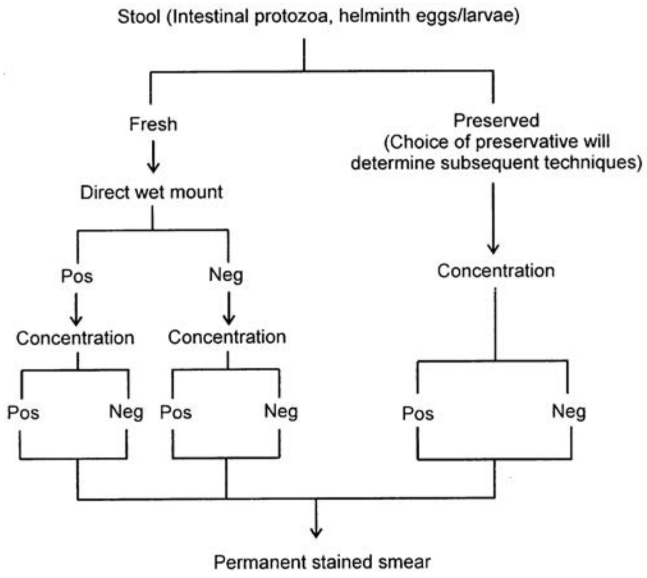


Figure 3.14 Dual-flow-cell Optical Slide Assembly (Apacor Ltd USA, Brooklyn, NY) that fits into the stage clips of any standard upright microscope. doi:10.1128/9781555819002.ch3.f14

2. After centrifugation (1 min at 500 × *g*), decant and discard the supernatant fluid. **Although stains for coccidian oocysts and microsporidian spores are not recommended from PVA-preserved material, the centrifuge speed indicated here is probably not sufficient to recover the oocysts or spores. Centrifugation time and speed are recommended as 10 min at 500 × *g*. The final amount of sediment should be about 0.5 to 1.0 ml. If necessary, adjust by repeating step 1 or by resuspending the sediment in saline (0.85% NaCl) and removing part of the suspension.**
3. Prepare a smear from the sediment for later staining by placing 1 drop of Mayer's albumin on the slide and adding 1 drop of SAF-preserved fecal sediment.

   Allow the smear to air dry at room temperature for 30 min prior to staining.
4. After being dried, the smear can be placed directly into 70% alcohol (step 4) of the staining procedure (the iodine-alcohol step can be eliminated).

Figure 3.15 Work flow diagram for fecal specimens. The total examination includes the direct mount, concentrate, and permanent stained smear (fresh specimen) or the concentrate and permanent stained smear (preserved specimens). doi:10.1128/9781555819002.ch3.f15


Stool (Intestinal protozoa, helminth eggs/larvae)
Fresh
Preserved (Choice of preservative will determine subsequent techniques)
Direct wet mount
Pos
Neg
Concentration
Concentration
Concentration
Pos
Neg
Pos
Neg
Pos
Neg
Permanent stained smear

Merthiolate-Iodine-Formalin (MIF)-Preserved Material

1. Prepare a Mayer's albumin-coated slide and allow it to air dry at room temperature for 30 min.
2. From the MIF vial (material allowed to settle in the vial for at least 1 h undisturbed), remove a portion of sediment and place this material onto the albumin-coated slide. Allow the slide to remain flat for 5 min; if there is still fluid left on the slide after this time, stand it up and allow the excess fluid to run off onto a paper towel.
3. After all excess fluid is removed, the slides are ready for staining in polychrome IV stain.

Universal Fixative (TOTAL-FIX)-Preserved Material

The TOTAL-FIX stool collection kit is a single-vial system that provides a standardized method for untrained personnel to properly collect and preserve stool specimens for the detection of helminth larvae and eggs, protozoan trophozoites and cysts, coccidian oocysts, and microsporidian spores. Concentrations, permanent stains, most fecal immunoassays, special stains, and some molecular testing can be performed from a TOTAL-FIX-preserved specimen. TOTAL-FIX is a mercury-, formalin-, and PVA-free fixative that preserves parasite morphology and helps with disposal and monitoring problems encountered by laboratories. The product contains zinc sulfate, acetic acid, and alcohols in water; however, the formula is proprietary and is patented (see Algorithm 3.1).

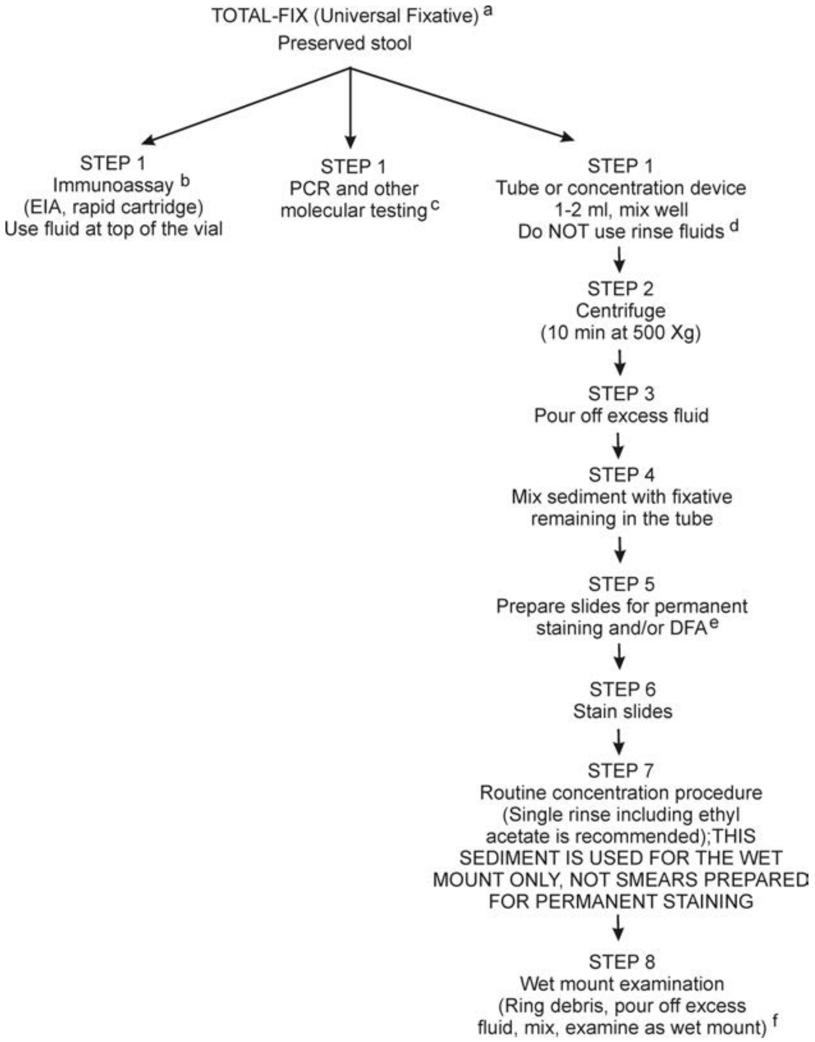
Trichrome Stain

The trichrome technique of Wheatley (22) for fecal specimens is a modification of Gomori's original staining procedure for tissue (7). It is a rapid, simple procedure which produces uniformly well-stained smears of the intestinal protozoa, human cells, yeast cells, and artifact material in about 45 min or less.

The specimen usually consists of fresh stool smeared on a microscope slide, which is immediately fixed in liquid Schaudinn's fixative, preserved stool (with or without PVA), the single-vial fixatives, or the Universal Fixative smeared on a slide and allowed to air dry.

Trichrome Stain

Chromotrope 2R	0.6 g
Light green SF	0.3 g
Phosphotungstic acid	0.7 g


TOTAL-FIX (Universal Fixative) a
Preserved stool
STEP 1
Immunoassay b
(EIA, rapid cartridge)
Use fluid at top of the vial
STEP 1
PCR and other
molecular testing c
STEP 1
Tube or concentration device
1-2 ml, mix well
Do NOT use rinse fluids d
STEP 2
Centrifuge
(10 min at 500 Xg)
STEP 3
Pour off excess fluid
STEP 4
Mix sediment with fixative
remaining in the tube
STEP 5
Prepare slides for permanent
staining and/or DFA e
STEP 6
Stain slides
STEP 7
Routine concentration procedure
(Single rinse including ethyl
acetate is recommended);THIS
SEDIMENT IS USED FOR THE WET
MOUNT ONLY, NOT SMEARS PREPARED
FOR PERMANENT STAINING
STEP 8
Wet mount examination
(Ring debris, pour off excess
fluid, mix, examine as wet mount) f

Algorithm 3.1 Method for the preparation of TOTAL-FIX-preserved fecal specimens (Universal Fixative) for the concentration, permanent stained smear, fecal immunoassays, and special stains for the coccidia and microsporidia. (a) This fixative contains NO mercury, NO formalin, and NO polyvinyl alcohol (PVA); use one-third stool and two-thirds fixative and MIX WELL. Allow 30 min prior to processing. (b) The EIA and rapid cartridge detect antigen; sample the clear fluid at the top of the vial containing the stool/fixative mixture. If the vial is shaken, make sure it stands on the counter for 5 to 10 min to allow the particulate material to settle out; work with the clear fluid only. (c) TOTAL-FIX has been tested with a number of molecular methods; however, there is no confirmation that the specimen will work with every possible available method. (d) Rinse fluids (water, saline, formalin) will PREVENT permanent stained smears from staining correctly; rinse fluids can ONLY be used for the final concentration step (after original sediment has been removed for permanent stained slide preparations—STEP 2). (e) Since the direct fluorescent antibody immunoassay (DFA) detects the actual *Giardia* cysts (rare trophozoites—much less intense fluorescence) and *Cryptosporidium* oocysts, this test sensitivity is enhanced if centrifuged sediment is used for testing; the same advantage is seen using modified acid-fast stains for *Cryptosporidium* (use sediment). Remember, do not use RINSED sediment, but sediment from the original centrifugation. (f) Examine entire 22-by-22-mm coverslip at low power (10× objective); examine one-third to one-half coverslip at high dry power (40× objective).

Acetic acid (glacial)	1.0 ml
Distilled water	100 ml

1. Prepare the stain by adding 1.0 ml of acetic acid to the dry components. Allow the mixture to stand (ripen) for 15 to 30 min at room temperature.
2. Add 100 ml of distilled water. Properly prepared stain is purple.
3. Store in a glass or plastic bottle at room temperature. The shelf life is 24 months.

70% Ethanol plus Iodine

1. Prepare a stock solution by adding iodine crystals to 70% alcohol until a dark solution is obtained (1 to 2 g/100 ml).
2. To use, dilute the stock solution with 70% alcohol until a dark reddish brown strong-tea color is obtained. As long as the color is acceptable, working solution does not have to be replaced. Replacement time depends on the number of smears stained and the size of the container (1 week to several weeks). **This dish is required ONLY if the fecal specimen has been preserved using a mercuric chloride-based fixative; it is not required for staining SAF-preserved fecal specimens.**

90% Ethanol, Acidified

90% ethanol	99.5 ml
Acetic acid (glacial)	0.5 ml

Prepare by combining.

70% Isopropyl or Ethyl Alcohol
100% Ethyl Alcohol (Recommended)
or 95%/5% Commercial Absolute Alcohol (Second Choice)

This is ethyl alcohol that has been denatured with isopropanol and methanol, but is considered commercial absolute alcohol and does not require a license to purchase (unlike absolute ethanol that has not been denatured). However, this product does not dehydrate as well as actual absolute ethanol.

Xylene or Xylene Substitute

Quality Control for Trichrome Stain

1. Stool samples used for QC can be fixed stool specimens known to contain protozoa or preserved negative stools to which buffy coat cells (PMNs or macrophages) have been added. A QC smear prepared from a positive fecal sample or a fixative sample containing buffy coat cells should be used when new stain is prepared or at least once each week. Cultured protozoa can also be used.
2. A QC slide should be included with a run of stained slides at least monthly; more frequent QC is recommended for those who may be unfamiliar with the method (14).
3. If the xylene becomes cloudy or there is an accumulation of water in the bottom of the staining dish containing xylene, discard the old reagents, clean the dishes, dry thoroughly, and replace with fresh 100% ethanol and xylene or xylene substitute.
4. All staining dishes should be covered to prevent evaporation of reagents (screw-cap Coplin jars or glass lids).
5. Depending on the volume of slides stained, staining solutions will have to be changed on an as-needed basis.
6. When the smear is thoroughly fixed and the staining procedure is performed correctly, the cytoplasm of protozoan trophozoites is blue-green, sometimes with a tinge of purple. Cysts tend to be slightly more purple. Nuclei and inclusions (chromatoidal bars, RBCs, bacteria, and Charcot-Leyden crystals) are red, sometimes tinged with purple. The background material usually stains green, providing a nice color contrast with the protozoa. This contrast is more distinct than that obtained with the hematoxylin stain, which tends to stain everything in shades of gray-blue to black.
7. If appropriate, the microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
8. Known positive microscope slides and photographs (reference books) should be available at the workstation.
9. Record all QC results; the laboratory should also have an action plan for "out-of-control" results.

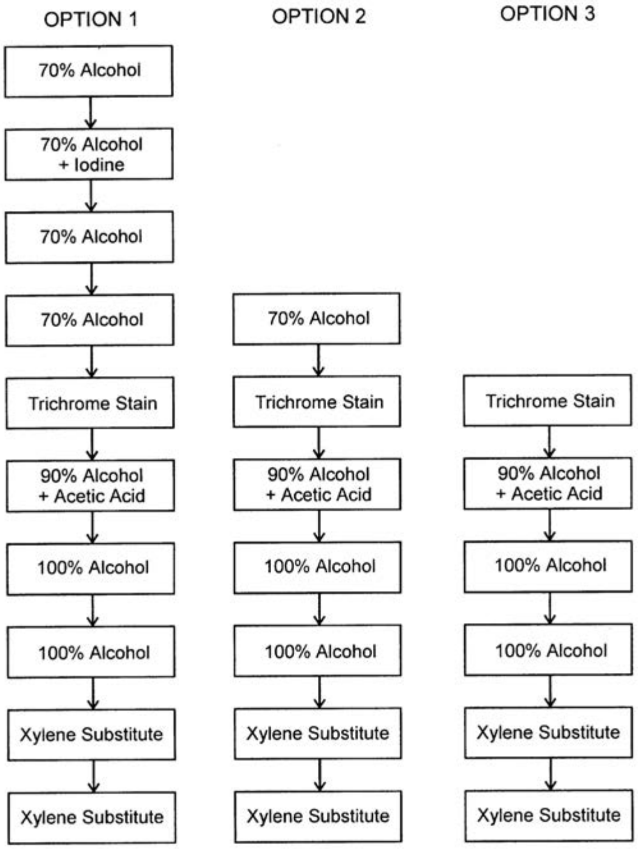

OPTION 1
OPTION 2
OPTION 3
70% Alcohol
70% Alcohol + Iodine
70% Alcohol
70% Alcohol
Trichrome Stain
90% Alcohol + Acetic Acid
100% Alcohol
100% Alcohol
Xylene Substitute
Xylene Substitute
70% Alcohol
Trichrome Stain
90% Alcohol + Acetic Acid
100% Alcohol
100% Alcohol
Xylene Substitute
Xylene Substitute
Trichrome Stain
90% Alcohol + Acetic Acid
100% Alcohol
100% Alcohol
Xylene Substitute
Xylene Substitute

Figure 3.16 Trichrome staining. Option 1, for use with smears prepared from fixatives containing mercuric chloride. The iodine is used to remove the mercuric chloride, and the subsequent two alcohol rinse steps remove the iodine. Thus, prior to staining, both the mercuric chloride and iodine have been removed from the smear. Options 2 and 3, for use with smears prepared from fixatives containing no mercuric chloride (the user can select option 2 or 3; there is minimal to no difference).
doi:10.1128/9781555819002.ch3.f16

Procedure for Trichrome Stain with Mercury-Based Fixatives (Fig. 3.16)

Note In all staining procedures for fecal and gastrointestinal tract specimens, the term "xylene" is used in the generic sense. Xylene substitutes are recommended for the safety of all personnel performing these procedures.

1. Prepare the slide for staining as described above.
2. Remove the slide from liquid Schaudinn's fixative, and place it in 70% ethanol for 5 min.
3. Place the slide in 70% ethanol plus iodine for 1 min for fresh specimens or 5 to 10 min for PVA air-dried smears. The exposure to iodine will remove the mercuric chloride from the smear prior to staining with the actual trichrome dyes (substitution of iodine for mercury).

4. Place the slide in 70% ethanol for 5 min.* This and the next step in 70% ethanol will remove the iodine from the smear.
5. Place it in a second container of 70% ethanol for 3 min.*
6. Place it in trichrome stain for 10 min. The fecal smear no longer contains either mercuric chloride or iodine and is now ready for staining.
7. Place it in 90% ethanol plus acetic acid for 1 to 3 s. Immediately drain the rack (see Procedure Notes), and proceed to the next step. Do not allow slides to remain in this solution. This is the destaining step.
8. Dip the slide several times in 100% ethanol. Use this step as a rinse.
9. Place it in two changes of 100% ethanol for 3 min each.* This is a dehydration step.
10. Place it in xylene or xylene substitute for 5 to 10 min.* This is a dehydration step.
11. Place it in a second container of xylene or xylene substitute for 5 to 10 min.* This step completes the dehydration process (removal of all water on the smear).
12. Mount the slide with a coverslip (no. 1 thickness), using mounting medium (e.g., Permount).
13. Allow the smear to dry overnight or after 1 h at 37°C.
14. Examine the smear microscopically with the 100× objective. Examine at least 200 to 300 oil immersion fields before reporting a negative result (Fig. 3.17).

*Slides may be held for up to 24 h in these solutions without harming the quality of the smear or stainability of organisms.

Procedure for Trichrome Stain with Non-Mercury-Based Fixatives (Iodine-Alcohol Step and Alcohol Rinse Not Required) (Fig. 3.16)

1. Prepare the slide for staining as described above.
2. Place the slide in 70% ethanol for 5 min.*
3. Place it in the trichrome stain for 10 min. Some people prefer to place the dry smear directly into the stain and eliminate step 2 from the protocol.
4. Place it in 90% ethanol plus acetic acid for 1 to 3 s. Immediately drain the rack (see Procedure Notes), and proceed to the next step. Do not allow slides to remain in this solution.

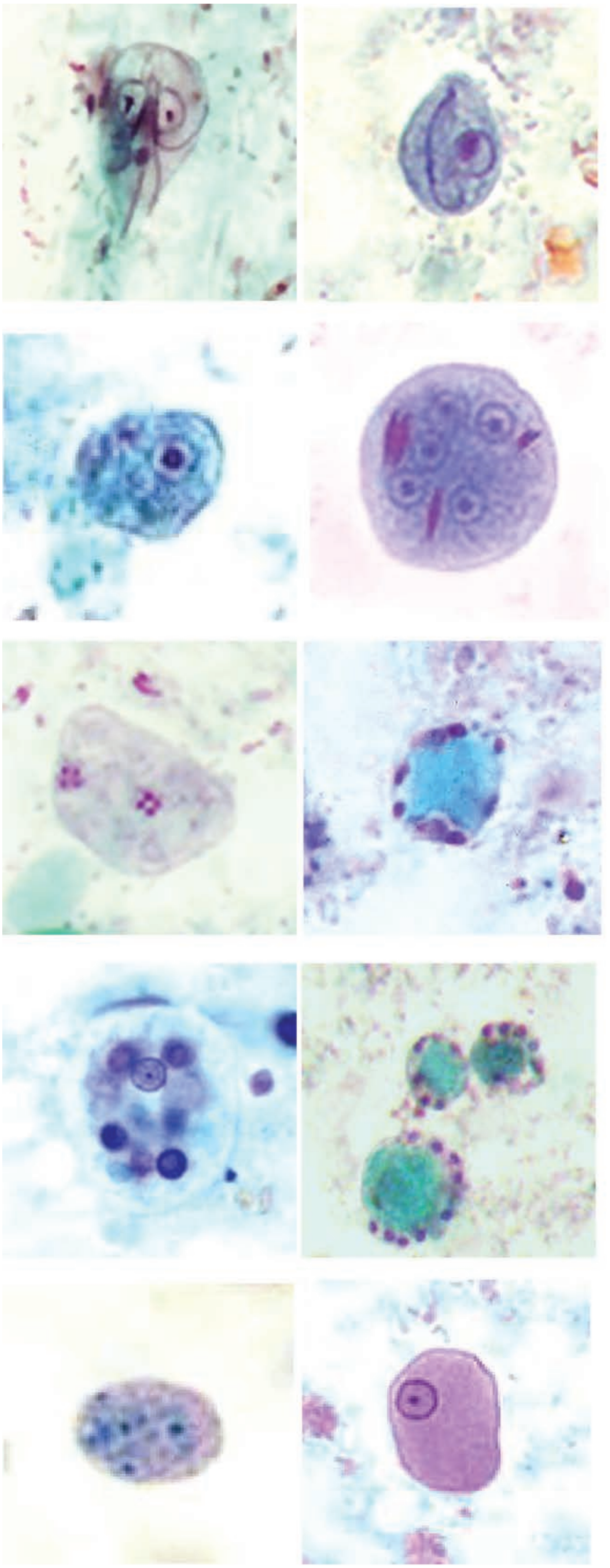


Figure 3.17 Guess the identity of these intestinal protozoa! See p. 76 for the answers. doi:10.1128/9781555819002.ch3.f17

5. Dip the slide several times in 100% ethanol. Use this step as a rinse.
6. Place it in two changes of 100% ethanol for 3 min each.*
7. Place it in xylene or xylene substitute for 5 to 10 min.*
8. Place it in a second container of xylene or xylene substitute for 5 to 10 min.*
9. Mount with a coverslip (no. 1 thickness), using mounting medium (e.g., Permount).
10. Allow the smear to dry overnight or after 1 h at 37°C.
11. Examine the smear microscopically with the 100× objective. Examine at least 200 to 300 oil immersion fields before reporting a negative result.

*Slides may be held for up to 24 h in these solutions without harming the quality of the smear or stainability of organisms.

Results and Patient Reports from the Trichrome Staining Method

Protozoan trophozoites and cysts are readily seen, although helminth eggs and larvae may not be easily identified because of excess stain retention (wet smears from the concentration procedure[s] are recommended for detection of these organisms) (Fig. 3.18 and 3.19). Yeasts (single and budding cells and pseudohyphae) and human cells (macrophages, PMNs, and RBCs) can be identified. The following quantitation chart can be used for examination of permanent stained smears with the oil immersion lens (100× objective; total magnification, ×1,000).

Quantitation of parasites, cells, yeasts, and artifacts

Quantity	No. per 10 oil immersion fields (×1,000)
Few	≤2
Moderate	3–9
Many	≥10

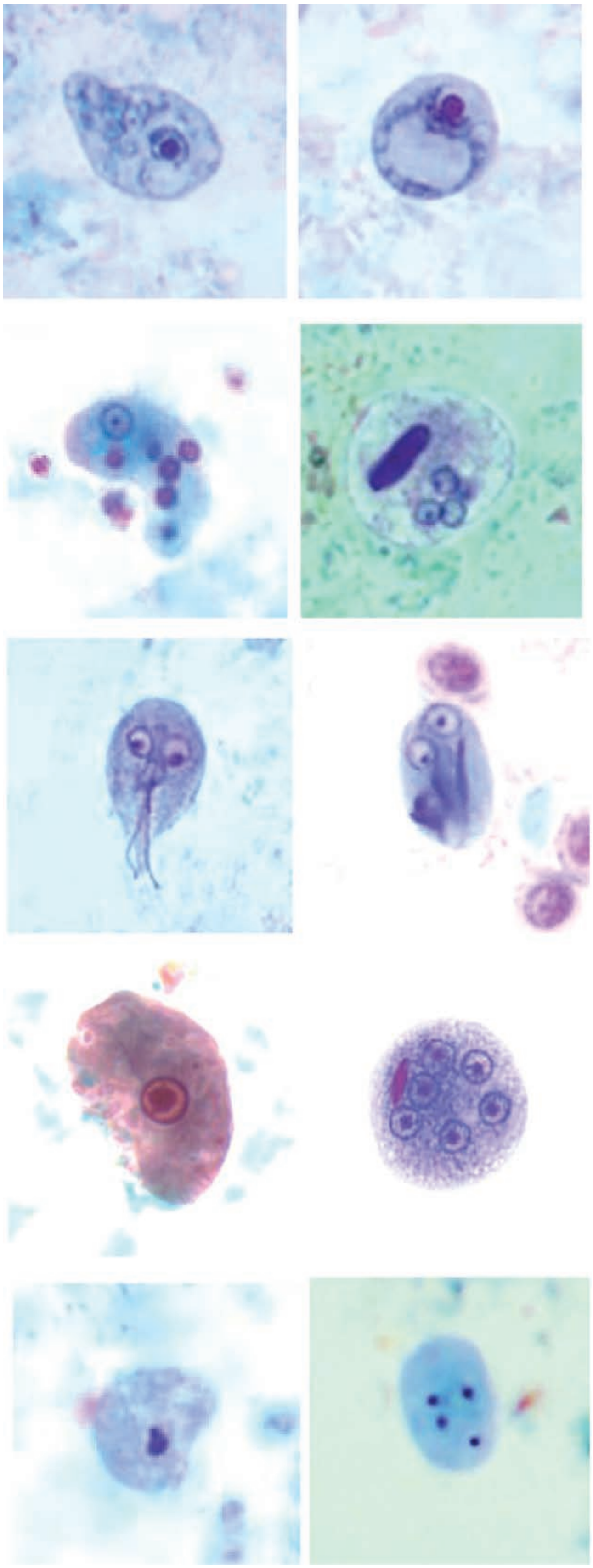


Figure 3.18 Intestinal protozoa (stained with Wheatley trichrome stain). Rows, from top to bottom (left and right), show *Iodamoeba bütschlii* trophozoite and *I. bütschlii cyst*; *Entamoeba histolytica* trophozoite (note the ingested RBCs) and *Entamoeba histolytica/E. dispar* cyst; *Giardia lamblia* (*G. duodenalis, G. intestinalis*) trophozoite and *G. lamblia* cyst; *Entamoeba coli* trophozoite and *E. coli* cyst; and *Endolimax nana* trophozoite and *E. nana* cyst. doi:10.1128/9781555819002.ch3.f18

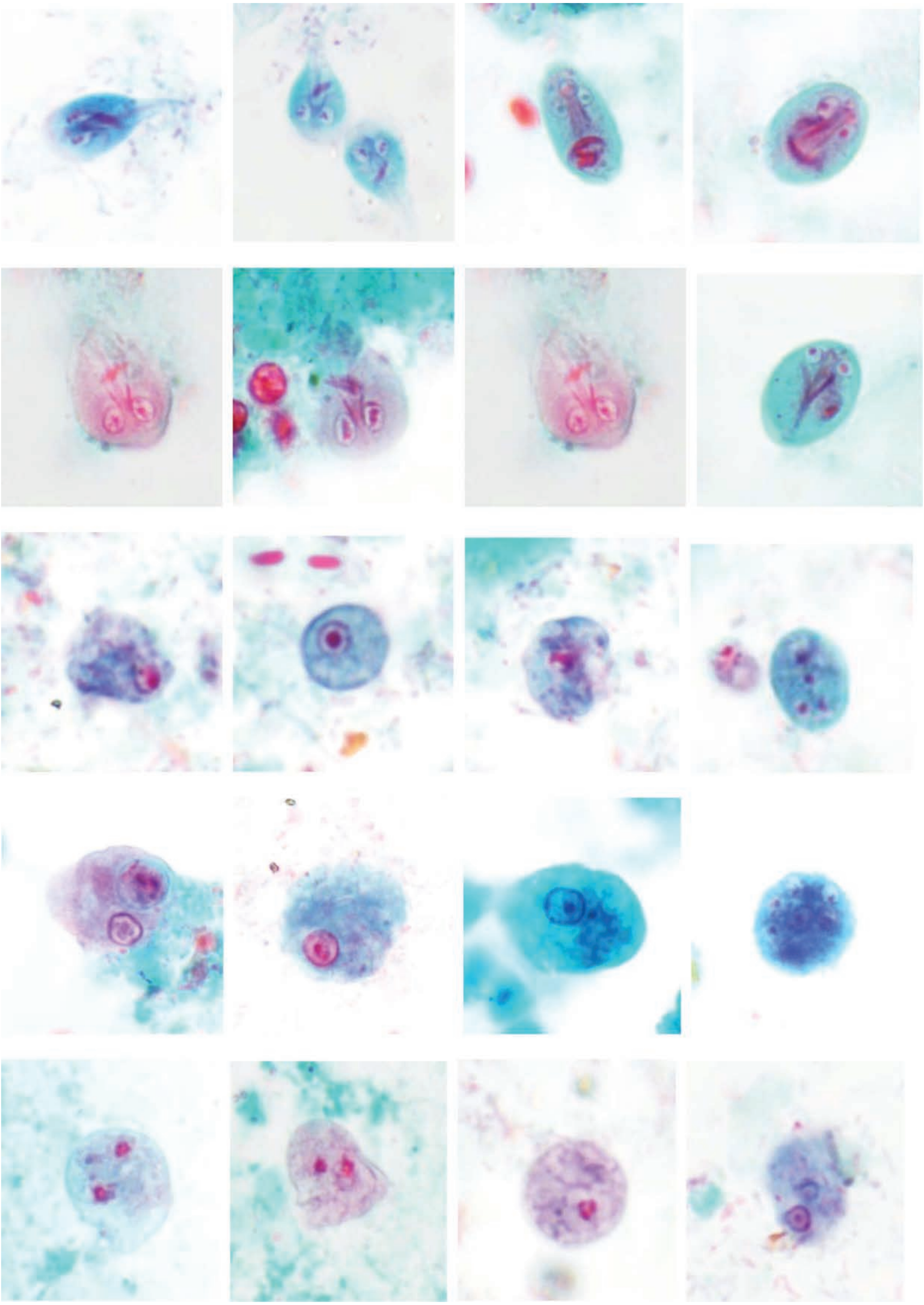
1. Report the organism and stage (do not use abbreviations).
   Examples: *Entamoeba histolytica/E. dispar* trophozoites
   Giardia lamblia (*G. duodenalis, G. intestinalis*) trophozoites
2. Quantitate the number of *Blastocystis* spp. organisms seen (rare, few, moderate, many). Do not quantitate other protozoa.
   Example: Moderate *Blastocystis* spp.
3. Note and quantitate the presence of human cells.
   Example: Moderate WBCs, many RBCs, few macrophages, rare Charcot-Leyden crystals
4. Report and quantitate yeast cells.
   Example: Moderate budding yeast cells and few pseudohyphae

   Note Because yeast can continue to grow if the stool is not immediately preserved, some laboratories do not report yeast, since the report can be misleading. They elect to call the physician and discuss the findings. Another option is to add a report comment indicating that reports of yeast (budding and/or pseudohyphae) might be misleading due to a lag time between stool passage and specimen fixation.
5. Save positive slides for future reference. Label prior to storage (name, patient number, organisms present). Most laboratories discard their negative permanent stained smears after several weeks (rotate storage boxes and discard when full).

Procedure Notes for the Trichrome Staining Method

1. The single most important step in the preparation of a well-stained fecal smear is good fixation. If this has not been done, the protozoa may be distorted or shrunk, may not be stained, or may exhibit an overall pink or red color with poor internal morphology.
2. Slides should always be drained between solutions. Touch the end of the slide to a paper towel for 2 s to remove excess fluid before proceeding to the next step. This will maintain the staining solutions for a longer period. The slide can also be touched against the staining dish to drain off excess fluid before moving on to the next dish.
3. Incomplete removal of mercuric chloride (Schaudinn's fixative with a mercuric chloride base [with or without PVA]) may cause the smear to contain highly refractive crystals or granules, which may prevent finding or identifying any organisms present. Since the 70% ethanol-iodine solution removes the mercury complex, it should be changed at least weekly to maintain the strong-tea color. A few minutes are usually sufficient to keep the slides in the iodine-alcohol; too long a time in this solution may also adversely affect the staining of the organisms.
4. When using non-mercury-based fixatives, the iodine-alcohol step (used for the removal of mercury) and the subsequent alcohol rinse (removal of the iodine) can be eliminated from the procedure. The smears for staining can be prerinsed with 70% alcohol and then placed in the trichrome stain, or they can be placed directly into the trichrome stain as the first step in the staining protocol (Fig. 3.16).
5. Smears that are predominantly green may be due to the inadequate removal of iodine by the 70% ethanol (steps 4 and 5). Lengthening the time of these steps or more frequent changing of the 70% ethanol will help.
6. To restore weakened trichrome stain, remove the cap and allow the ethanol to evaporate (ethanol carried over on the staining rack from a previous dish). After a few hours, fresh stock stain may be added to restore lost volume. Older, more concentrated stain produces more intense colors and may require slightly longer destaining times (an extra dip). Smears from fixatives containing PVA usually require a slightly longer staining time due to the presence of the plastic PVA powder.
7. Although the trichrome stain is used essentially as a "progressive" stain (that is, no destaining is necessary), best results are obtained by using the stain "regressively" (destaining the smears briefly in acidified alcohol after the initial overstaining). Good differentiation is obtained by destaining for a very short time (two dips only, approximately 2 to 3 s); prolonged destaining results in poor differentiation.
8. It is essential to rinse the smears free of acid to prevent continued destaining. Since 90% alcohol will continue to leach trichrome stain from the smears, it is recommended that after the acid-alcohol is used, the slides be quickly rinsed in 100% alcohol and then dehydrated through two additional changes of 100% alcohol.

Figure 3.19 Intestinal protozoa (preserved with TOTAL-FIX, stained with Wheatley trichrome stain). From top to bottom and left to right. Top row: *Giardia lamblia* (*G. duodenalis, G. intestinalis*), 2 trophozoites, 2 cysts. Second row: *Giardia lamblia* (*G. duodenalis, G. intestinalis*), 3 trophozoites, 1 cyst. Third row: *Endolimax nana*, 3 trophozoites, 1 cyst. Note the nuclear variation in the three trophozoites (very common). Fourth row: *Entamoeba coli*, 3 trophozoites and 1 cyst. Fifth row: *Dientamoeba fragilis* (3 trophozoites) and *Entamoeba hartmanni* (1 trophozoite). doi:10.1128/9781555819002.ch3.f19



9. In the final stages of dehydration (steps 9 to 11), the 100% ethanol and the xylenes (or xylene substitute) should be kept as free from water as possible. Coplin jars must have tight-fitting caps to prevent both evaporation of reagents and absorption of moisture. If the xylene becomes cloudy after addition of slides from the 100% ethanol, return the slides to fresh 100% ethanol and replace the xylene with fresh stock.
10. If the smears peel or flake off, the specimen might have been inadequately dried on the slide (in the case of specimens containing PVA), the smear may have been too thick, or the slide may have been greasy (fingerprints). However, slides generally do not have to be cleaned with alcohol prior to use.
11. On examination, if the stain appears unsatisfactory and it is not possible to obtain another slide to stain, the slide may be restained. Place the slide in xylene to remove the coverslip, and reverse the dehydration steps, adding 50% ethanol as the last step. Destain the slide in 10% acetic acid for several hours, and then wash it thoroughly first in water and then in 50 and 70% ethanol. Place the slide in the trichrome stain for 8 min, and complete the staining procedure (3).

Procedure Limitations for the Trichrome Staining Method

1. The permanent stained smear is not recommended for staining helminth eggs or larvae; they are often too dark (excess stain retention) or distorted. However, they are occasionally recognized and identified. The wet smear preparation from the concentrate is the recommended approach for identification of helminth eggs and larvae.
2. The smear should be examined with the oil immersion lens (100×) for the identification of protozoa, human cells, Charcot-Leyden crystals, yeast cells, and artifact material. Quantitation of these cells and other structures is normally done from the examination of the permanent stained smear, not the wet smear preparations (direct wet smear or concentration wet smear).
3. This high-magnification (oil immersion; total magnification, ×1,000) examination is recommended for protozoa, particularly for confirming species identification.
4. With low magnification (10× objective), one might see eggs or larvae; however, this is not recommended as a routine approach.
5. In addition to helminth eggs and larvae, *C. belli* oocysts are best seen in wet preparations (concentration wet smears prepared from formalin-preserved, not PVA-preserved, material).
6. *Cryptosporidium* and *Cyclospora* oocysts are generally not recognized on a trichrome-stained smear (modified acid-fast stains or the immunoassay reagent kits are recommended). Microsporidial spores do not stain sufficiently for recognition by the regular trichrome method; modified trichrome stains are required.

Trichrome Stain (Modified for Use with SAF-Preserved Fecal Specimens) (Dr. Norbert Ryan, VIDRL Modification)

It is generally recognized that stained fecal films are the single most productive means of stool examination for intestinal protozoa. The permanent stained smear facilitates detection and identification of cysts and trophozoites and affords a permanent record of the protozoa encountered. Small protozoa missed by direct smear and concentration techniques are often seen on the stained smear. Many reference texts mention that the combination of trichrome and SAF is unsuitable, because the green background stain of trichrome dominates, perhaps interacting with the albumin used to bind material to the slide. In this method the trichrome stain has been modified to reduce background staining. The modification is based on the theory that phosphotungstic acid functions both as a mordant and as a "colorless" stain of medium size. By increasing the content of this agent the intensity of background staining is reduced, without compromising the staining of structures with small pore size such as protozoa and tissue cells.

Note All other sections related to the trichrome stain presented above are relevant for the VIDRL modification of the trichrome stain for SAF-preserved fecal specimens presented here.

Trichrome Stain (VIDRL Modification for SAF-fixed smears)

Chromotrope 2R	0.6 g
Light green SF	0.3 g
Phosphotungstic acid	1.0 g
Acetic acid (glacial)	1.0 ml
Distilled water	100 ml

1. Prepare the stain by adding 1.0 ml of acetic acid to the dry components. Allow the mixture to stand (ripen) for 15 to 30 min at room temperature.
2. Add 100 ml of distilled water. Properly prepared stain is purple.
3. Store in a glass or plastic bottle at room temperature. The shelf life is 24 months.

Procedure for Trichrome Stain (VIDRL Modification)

Note In all staining procedures for fecal and gastrointestinal tract specimens, the term "xylene" is used in the generic sense. Xylene substitutes are recommended for the safety of all personnel performing these procedures.

1. Place the slide in 70% ethanol for 5 min.*
2. Place it in a second container of 70% ethanol for 3 min.*
3. Place it in trichrome stain (VIDRL modification) for 10 min. The fecal smear no longer contains either mercuric chloride or iodine and is now ready for staining.
4. Place it in 90% ethanol plus 0.5% acetic acid for 1 to 3 s. Immediately drain the slide (see Procedure Notes), and proceed to the next step. Do not allow slides to remain in this solution. This is the destaining step.
5. Place the slide in 95% ethanol for 1 to 3 s. Use this step as a rinse.
6. Place it in two changes of 100% ethanol for 3 min each.* This is a dehydration step.
7. Place it in xylene or xylene substitute for 10 min.* This is a dehydration step.
8. Mount the slide with a coverslip (no. 1 thickness), using mounting medium (e.g., Permount).
9. Allow the smear to dry overnight or after 1 h at 37°C.
10. Examine the smear microscopically with the 100× objective. Examine at least 200 to 300 oil immersion fields before reporting a negative result (Fig. 3.17).

*Slides may be held for up to 24 h in these solutions without harming the quality of the smear or stainability of organisms.

Note An alternative method to using mounting medium is as follows (2).

A. Remove the slide from the last container of xylene, place it on a paper towel (flat position), and allow it to air dry. Remember that some of the xylene substitutes may take a bit longer to dry than xylene itself does.

B. Approximately 5 to 10 min before you want to examine the slide, place a drop of immersion oil on the dry fecal film. Allow the oil to sink into the film for a minimum of 10 to 15 min. If the smear appears to be very refractile on examination, you have not waited long enough for the oil to sink into the film or you need to add a bit more oil to the film.

C. Once you are ready to examine the slide, place a no. 1 (22- by 22-mm) coverslip onto the oiled smear, add another drop of immersion oil onto the top of the coverslip (as you would normally do for any slide with a coverslip), and examine with the oil immersion lens (100× objective).

D. *Do not eliminate adding the coverslip;* the dry fecal material on the slide often becomes very brittle after dehydration. Without the addition of the protective coverslip, you might scratch the surface of the oil immersion lens. Coverslips are much cheaper than oil immersion objectives!

Iron Hematoxylin Stain

The iron hematoxylin stain is one of a number of stains that allow one to make a permanent stained slide for detecting and quantitating parasitic organisms. Iron hematoxylin was the stain used for most of the original morphologic descriptions of intestinal protozoa found in humans (3) (Fig. 3.20). On oil immersion power (×1,000), one can examine the diagnostic features used to identify the protozoan parasites. Although there are many modifications of iron hematoxylin techniques, only two methods are outlined below: the Spencer-Monroe (19) and Tompkins-Miller (20) procedures. Both methods can be used with either fresh specimens or those preserved with SAF, preservatives containing PVA, single-vial systems, or the Universal Fixatives.

The specimen usually consists of fresh stool smeared on a microscope slide, which is immediately fixed in Schaudinn's fixative; stool in fixative containing PVA smeared on a slide and allowed to air dry; SAF-preserved stool smeared on an albumin-coated slide and allowed to air dry; single-vial-preserved stool smeared on an albumin-coated slide and allowed to air dry; or stool smeared on a slide and allowed to air dry (Universal Fixative, no adhesive is required).

Iron Hematoxylin Stain (Spencer-Monroe Method) (19)

Solution 1

Hematoxylin (crystal or powder)	10 g
Ethanol (absolute)	1,000 ml

Place solution in a stoppered clear flask or bottle, and allow it to ripen in a lighted room for at least 1 week at room temperature.

Solution 2

Ferrous ammonium sulfate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$	10 g
Ferric ammonium sulfate $[FeNH_4(SO_4)_2 \cdot 12H_2O]$	10 g
Hydrochloric acid (concentrated)	10 ml
Distilled water	1,000 ml

Working Solution

Mix equal volumes of solutions 1 and 2. The working solution should be made fresh every week.

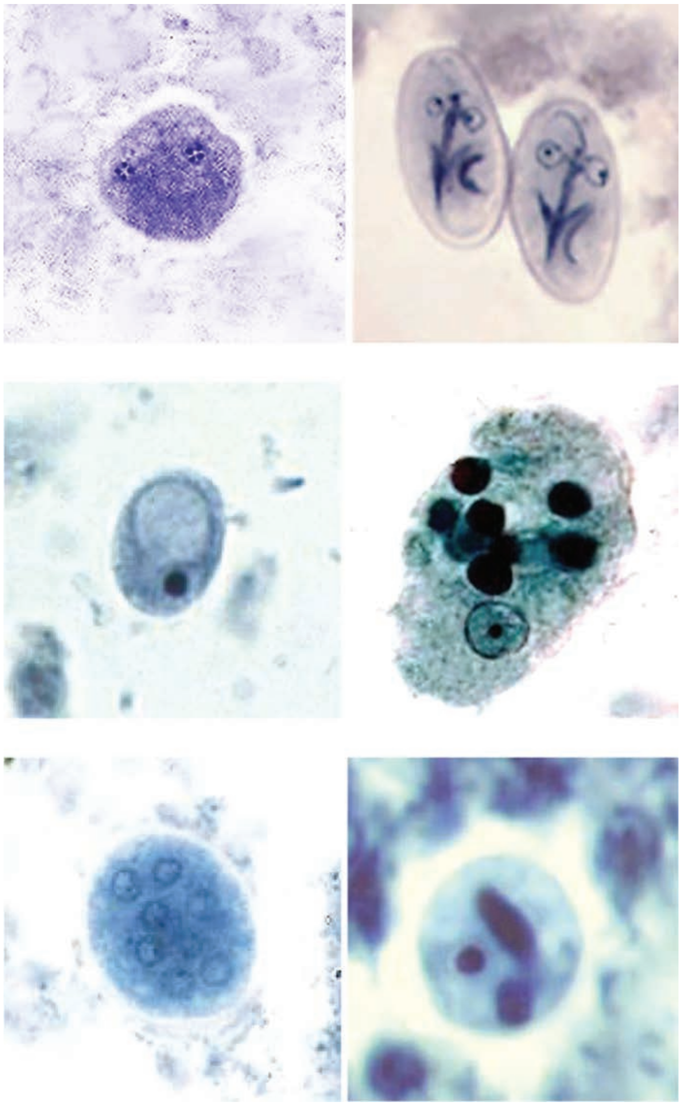


Figure 3.20 Intestinal protozoa stained with iron hematoxylin stain. (Top row) *Dientamoeba fragilis* trophozoite (left), *Giardia lamblia* (*G. duodenalis, G. intestinalis*) cysts (right); (middle row) *Iodamoeba bütschlii* cyst (note the large glycogen vacuole) (left), *Entamoeba histolytica* trophozoite (note ingested RBCs) (right); (bottom row) *Entamoeba coli* cyst (note more than five nuclei) (left), *Entamoeba histolytica/E. dispar* cyst (right). doi:10.1128/9781555819002.ch3.f20

70% Ethanol plus Iodine

1. Prepare a stock solution by adding iodine crystals to 70% alcohol until a dark solution is obtained (1 to 2 g/100 ml).
2. To use, dilute the stock solution with 70% alcohol until a dark reddish brown strong-tea color is obtained. As long as the color is acceptable, new working solution does not have to be made. The replacement time will depend on the number of smears stained and the size of the container (1 week to several weeks).

90% Ethanol, Acidified

90% Ethanol	99.5 ml
Acetic acid (glacial)	0.5 ml

Prepare by combining.

70% Isopropyl or Ethyl Alcohol

100% Ethyl Alcohol (Recommended)

or 95%/5% Commercial Absolute Alcohol (Second Choice)

This is ethyl alcohol that has been denatured with isopropanol and methanol, but is considered commercial absolute alcohol and does not require a license to purchase (unlike absolute ethanol that has not been denatured). However, this product does not dehydrate as well as actual absolute ethanol.

Xylene or Xylene Substitute

Quality Control for Iron Hematoxylin Stain

1. Stool samples used for quality control can be fixed stool specimens known to contain protozoa or preserved negative stools containing PVA to which buffy coat cells (PMNs or macrophages) have been added. A QC smear prepared from a positive sample or a sample containing buffy coat cells should be used when new stain is prepared or at least once each week. Cultured protozoa can also be used.
2. A QC slide should be included with a run of stained slides at least monthly; more frequent QC is recommended for those who may be unfamiliar with the method.
3. If the xylene or xylene substitute becomes cloudy or there is an accumulation of water in the bottom of the staining dish, discard the old reagents, clean the dishes, dry them thoroughly, and replace with fresh 100% ethanol and xylene or xylene substitute.
4. All staining dishes should be covered to prevent evaporation of reagents (screw-cap Coplin jars or glass lids).
5. Depending on the volume of slides stained, staining solutions should to be changed on an as-needed basis.
6. When the smear is thoroughly fixed and the stain is performed correctly, the cytoplasm of protozoan trophozoites will be blue-gray, sometimes with a tinge of black. Cysts tend to be slightly darker. Nuclei and inclusions (chromatoidal bars, RBCs, bacteria, and Charcot-Leyden crystals) are dark gray-blue, sometimes almost black. The background material usually stains pale gray or blue,

providing some color intensity contrast with the protozoa. This contrast is less distinct than that obtained with the trichrome stain, which tends to stain everything with multiple colors (pink, red, purple, green, blue).

7. The microscope should be calibrated (within the last 12 months) (recommended but not always required, depending on the use and care of the microscope), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
8. Known positive microscope slides and photographs (reference books) should be available at the workstation.
9. Record all QC results; the laboratory should also have an action plan for "out-of-control" results.

Procedure for Iron Hematoxylin Stain with Mercury-Based Fixatives

Note In all staining procedures for fecal and gastrointestinal tract specimens, the term "xylene" is used in the generic sense. Xylene substitutes are recommended for the safety of all personnel performing these procedures.

1. Prepare the slide for staining as previously described (for SAF smears or smears prepared from other non-mercury single-vial preservatives, proceed to step 4).
2. Place the slide in 70% ethanol for 5 min.
3. Place the slide in the iodine-70% ethanol (70% alcohol to which is added enough D'Antoni's iodine to obtain a strong-tea color) solution for 2 to 5 min. The iodine is designed to remove the mercury from the smear.
4. Place it in 70% ethanol for 5 min (this rinse step removes the iodine). Begin the procedure for SAF-fixed slides at this point.*
5. Wash the slide in running tap water (constant stream of water into the container) for 10 min.
6. Place the slide in iron hematoxylin working solution for 4 to 5 min.
7. Wash the slide in running tap water (constant stream of water into the container) for 10 min.
8. Place the slide in 70% ethanol for 5 min.*
9. Place the slide in 95% ethanol for 5 min.*
10. Place the slide in two changes of 100% ethanol for 5 min each.*
11. Place the slide in two changes of xylene for 5 min each.*
12. Add Permount to the stained area of the slide, and cover with a coverslip.

    Note An alternative method to using mounting medium is given on p. 53.
13. Examine the smear microscopically with the 100× objective. Examine at least 200 to 300 oil immersion fields before reporting a negative result.

*Slides may be held for up to 24 h in these solutions without harming the quality of the smear or stainability of organisms.

Procedure for Iron Hematoxylin Stain with Non-Mercury-Based Fixatives

1. Prepare the slide for staining as described above.
2. Place it in 70% ethanol for 5 min.*
3. Wash the slide in running tap water (constant stream of water into the container) for 10 min.
4. Place the slide in iron hematoxylin working solution for 4 to 5 min.
5. Wash the slide in running tap water (constant stream of water into the container) for 10 min.
6. Place the slide in 70% ethanol for 5 min.*
7. Place the slide in 95% ethanol for 5 min.*
8. Place the slide in two changes of 100% ethanol for 5 min each.*
9. Place the slide in two changes of xylene for 5 min each.*
10. Add Permount to the stained area of the slide and cover it with a coverslip.

    Note An alternative method to using mounting medium is given on p. 53.
11. Examine the smear microscopically with the 100× objective. Examine at least 200 to 300 oil immersion fields before reporting a negative result.

*Slides may be held for up to 24 h in these solutions without harming the quality of the smear or stainability of organisms.

Results and Patient Reports from the Iron Hematoxylin Staining Method

Protozoan trophozoites and cysts are readily visible, although helminth eggs and larvae may not be easily identified because of excess stain retention (wet smears from the concentration procedure[s] are recommended for detection of these organisms). Yeasts (single and budding cells and pseudohyphae) and human cells (macrophages, PMNs, and RBCs) can be identified. The following quantitation chart can be used for examination of permanent stained smears with the oil immersion lens (100× objective; total magnification, ×1,000).

Quantitation of parasites, cells, yeasts, and artifacts

Quantity	No. per 10 oil immersion fields (×1,000)
Few	≤2
Moderate	3–9
Many	≥10

1. Report the organism and stage (do not use abbreviations).
   Examples: *Entamoeba coli* trophozoites
   Dientamoeba fragilis trophozoites
2. Quantitate the number of *Blastocystis* spp. organisms seen (rare, few, moderate, many). Do not quantitate other protozoa.
   Example: Many *Blastocystis* spp.
3. Note and quantitate the presence of human cells.
   Example: Moderate WBCs, few macrophages, few RBCs, rare Charcot-Leyden crystals
4. Report and quantitate yeast cells.
   Example: Many budding yeast cells and few pseudohyphae

   NOTE Because yeast can continue to grow if the stool is not immediately preserved, some laboratories do not report yeast, since the report can be misleading. They elect to call the physician and discuss the findings. Another option is to add a report comment indicating that reports of yeast (budding and/or pseudohyphae) might be misleading due to a lag time between stool passage and specimen fixation.
5. Save positive slides for future reference. Label prior to storage (name, patient number, organisms present). Most laboratories hold their negative smears for several weeks and then discard them; slide boxes that are rotated can be used to batch, store, and discard negative smears.

Procedure Notes for the Iron Hematoxylin Staining Method

1. The single most important step in the preparation of a well-stained fecal smear is good fixation. If this has not been done, the protozoa may be distorted or shrunk, may not be stained, or may exhibit an overall gray or blue-gray color with poor internal morphology.
2. Slides should always be drained between solutions. Touch the end of the slide to a paper towel for 2 s to remove excess fluid before proceeding to the next step. This will maintain the staining solutions for a longer period. The slides can also be drained against the edge of each container before being moved to the next container.
3. Incomplete removal of mercuric chloride (Schaudinn's fixative and PVA fixative prepared with a mercuric chloride base) may cause the smear to contain highly refractive crystals or granules, which may prevent finding or identifying any organisms present. Since the 70% ethanol–iodine solution removes the mercury complex, it should be changed at least weekly to maintain the strong-tea color. A few minutes are usually sufficient to keep the slides in the iodine-alcohol; too long a time in this solution may also adversely affect the staining of the organisms.
4. When using non-mercury-based fixatives, the iodine-alcohol step (used for the removal of mercury) and the subsequent alcohol rinse (used for the removal of iodine) can be eliminated from the procedure. The smears for staining can be prerinsed with 70% alcohol and then placed in the water step prior to the hematoxylin stain as the first step in the staining protocol.
5. For staining large numbers of slides, the working hematoxylin solution may be diluted and affect the quality of the stain. If dilution occurs, discard the working solution and prepare a fresh working solution.
6. The shelf life of the stock hematoxylin solutions may be extended by keeping the solutions in the refrigerator at 4°C. Because of crystal formation in the working solutions, it may be necessary to filter them before preparing a new working solution.
7. In the final stages of dehydration (steps 9 to 11), the 100% ethanol and the xylenes should be kept as free from water as possible. Coplin jars must have tight-fitting caps to prevent both evaporation of reagents and absorption of moisture. If the xylene becomes cloudy after addition of slides from the 100% ethanol, return the slides to fresh 100% ethanol and replace the xylene with fresh stock.
8. If the smears peel or flake off, the specimen might have been inadequately dried on the slide (in the case of fixed specimens containing PVA), the smear may have been too thick, or the slide may have been greasy (fingerprints). However, slides generally do not have to be cleaned with alcohol prior to use.
9. On examination, if the stain appears unsatisfactory and it is not possible to obtain another slide to stain, the slide may be restained. Place the slide in xylene to remove the coverslip, and reverse the dehydration steps, adding 50% ethanol as the last step. Destain the slide in 10% acetic acid for several hours, and then wash it thoroughly first in water

and then in 50 and 70% ethanol. Place the slide in the iron hematoxylin stain for 8 min, and complete the staining procedure (2, 3).

Procedure Limitations for the Iron Hematoxylin Staining Method

1. The permanent stained smear is not recommended for staining helminth eggs or larvae; these structures are often too dark (excess stain retention) or distorted. However, they are occasionally recognized and identified. The wet smear preparation from the concentrate is the recommended approach for identification of helminth eggs and larvae.
2. The smear should be examined with the oil immersion lens (100×) for the identification of protozoa, human cells, Charcot-Leyden crystals, yeast cells, and artifact material. Quantitation of these cells and other structures is normally done from the examination of the permanent stained smear, not the wet smear preparations (direct wet smear, concentration wet smear).
3. This high-magnification (oil immersion; total magnification, ×1,000) examination is recommended for protozoa, particularly for confirming species identification.
4. With low magnification (10× objective), one might see eggs or larvae; however, this is not recommended as a routine approach.
5. In addition to helminth eggs and larvae, *C. belli* oocysts are best seen in wet preparations (concentration wet smears prepared from formalin-preserved, not preserved stool containing PVA).
6. *Cryptosporidium* and *Cyclospora* oocysts are generally not recognized on an iron hematoxylin-stained smear (modified acid-fast stains or the fecal immunoassay for *Cryptosporidium* spp. is recommended).

Iron Hematoxylin Stain (Tompkins-Miller Method) (20)

A longer iron hematoxylin method was described by Tompkins and Miller (20). Since differentiation of overstained slides is critical in most iron hematoxylin staining procedures, Tompkins and Miller have described a method that employs phosphotungstic acid to destain the protozoa and that gives excellent results, even in unskilled hands.

1. Prepare the slide for staining as described above (for SAF or non-mercury-based smears, proceed to step 4).
2. Place the slide in 70% ethanol for 5 min.
3. Place the slide in the iodine–70% ethanol (70% alcohol to which is added enough D'Antoni's iodine to obtain a strong-tea color) solution for 2 to 5 min.
4. Place it in 50% ethanol for 5 min. Begin the procedure for SAF- or non-mercury-fixed slides at this point.*
5. Wash the slide in running tap water (constant stream of water into the container) for 3 min.
6. Place the slide in 4% ferric ammonium sulfate mordant for 5 min.
7. Wash the slide in running tap water (constant stream of water into the container) for 1 min.
8. Place the slide in 0.5% aqueous hematoxylin for 2 min.
9. Wash the slide in tap water for 1 min.
10. Place the slide in 2% phosphotungstic acid for 2 to 5 min.
11. Wash the slide in running tap water for 10 min.
12. Place the slide in 70% ethanol (plus a few drops of saturated aqueous lithium carbonate) for 3 min.
13. Place the slide in 95% ethanol for 5 min.*
14. Place the slide in two changes of 100% ethanol for 5 min each.*
15. Place the slide in two changes of xylene for 5 min each.*
16. Add Permount to the stained area of the slide, and cover it with a coverslip.

Note An alternative method to using mounting medium is as follows.

A. Remove the slide from the last container of xylene, place it on a paper towel (flat position), and allow it to air dry. Remember that some of the xylene substitutes may take a bit longer to dry.
B. Approximately 5 to 10 min before you want to examine the slide, place a drop of immersion oil on the dry fecal film. Allow the oil to sink into the film for a minimum of 10 to 15 min. If the smear appears to be very refractile on examination, you have not waited long enough for the oil to sink into the film or you need to add a bit more oil onto the film.
C. Once you are ready to examine the slide, place a no. 1 (22- by 22-mm) coverslip onto the oiled smear, add another drop of immersion oil to the top of the coverslip (as you would normally do for any slide with a coverslip), and examine with the oil immersion lens (100× objective).
D. *Do not eliminate adding the coverslip;* the dry fecal material on the slide often becomes very brittle after dehydration. Without the addition of the protective coverslip, you might scratch

the surface of your oil immersion lens. Coverslips are much cheaper than oil immersion objectives!

17. Examine the smear microscopically with the 100× objective. Examine at least 200 to 300 oil immersion fields before reporting a negative result.

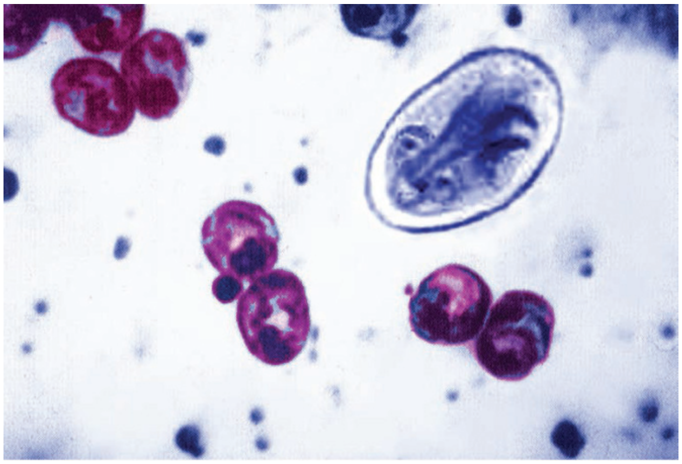
*Slides may be held for up to 24 h in these solutions without harming the quality of the smear or stainability of organisms.

Modified Iron Hematoxylin Stain (Incorporating the Carbol Fuchsin Step)

The following combination staining method for SAF-preserved fecal specimens was developed to allow the microscopist to screen for acid-fast organisms in addition to other intestinal parasites (Fig. 3.21). For laboratories using iron hematoxylin stains in combination with SAF-fixed material and modified acid-fast stains for *Cryptosporidium*, *Cyclospora*, and *Cystoisospora,* this modification represents another staining option (49). This combination stain provides a saving in both time and personnel use. However, some laboratories prefer to use these two stains as single methods; the overall result may be better than the combination approach. The selection of one approach or the other would be up to the laboratory and would be based on personal preferences.

Any fecal specimen submitted in SAF, other non-mercury single-vial-system preservatives, or the Universal Fixative can be used. Fresh fecal specimens after fixation in SAF for 30 min can also be used. This combination stain approach is not recommended for specimens preserved in Schaudinn's fixative using a mercuric chloride base (with or without PVA).

Figure 3.21 Iron hematoxylin stain incorporating the carbol fuchsin step. Note the modified acid-fast *Cryptosporidium* oocysts (4–6 µm) and the *Giardia lamblia* cyst.
doi:10.1128/9781555819002.ch3.f21



Mayer's Albumin

Add an equal quantity of glycerin to a fresh egg white. Mix gently and thoroughly. Store at 4°C, and indicate an expiration date of 3 months. Mayer's albumin from commercial suppliers can normally be stored at 25°C for 1 year.

Stock Solution of Hematoxylin Stain

Hematoxylin powder	10 g
Ethanol (95% or 100%)	1,000 ml

1. Mix well until dissolved.
2. Store in a clear-glass bottle in a light area. Allow to ripen for 14 days before use.
3. Store at room temperature with an expiration date of 1 year.

Mordant

Ferrous ammonium sulfate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$	10 g
Ferric ammonium sulfate $[FeNH_4(SO_4)_2 \cdot 12H_2O]$	10 g
Hydrochloric acid (concentrated)	10 ml
Distilled water to 1,000 ml	

Working Solution of Hematoxylin Stain

1. Mix equal quantities of stock solution of stain and mordant.
2. Allow the mixture to cool thoroughly before use (prepare at least 2 h prior to use). The working solution should be made fresh every week.

Picric Acid

Mix equal quantities of distilled water and an aqueous saturated solution of picric acid to make a 50% saturated solution.

Acid-Alcohol Decolorizer

Hydrochloric acid (concentrated)	30 ml
Alcohol to	1,000 ml

70% Alcohol and Ammonia

70% Alcohol	50 ml
Ammonia	0.5–1.0 ml

Add enough ammonia to bring the pH to approximately 8.0.

Carbol Fuchsin

1. To make basic fuchsin (solution A), dissolve 0.3 g of basic fuchsin in 10 ml of 95% ethanol.
2. To make phenol (solution B), dissolve 5 g of phenol crystals in 100 ml of distilled water. (Gentle heat may be needed.)

3. Mix solution A with solution B.
4. Store at room temperature. The solution is stable for 1 year.

Procedure for Modified Iron Hematoxylin Stain (Carbol Fuchsin Step)

1. Prepare slide.
    - **A.** Place 1 drop of Mayer's albumin on a labeled slide.
    - **B.** Thoroughly mix the sediment from the fecal concentration with an applicator stick.
    - **C.** Add approximately 1 drop of the fecal concentrate to the albumin, and spread the mixture over the slide.
2. Allow the slide to air dry at room temperature (the smear appears opaque when dry).
3. Place the slide in 70% alcohol for 5 min.
4. Wash the slide in a container of tap water (not under running water) for 2 min.
5. Place the slide in Kinyoun's stain for 5 min.
6. Wash the slide in running tap water (constant stream of water into container) for 1 min.
7. Place the slide in acid-alcohol decolorizer for 4 min.*
8. Wash the slide in running tap water (constant stream of water into container) for 1 min.
9. Place the slide in iron hematoxylin working solution for 8 min.
10. Wash the slide in distilled water (in container) for 1 min.
11. Place the slide in picric acid solution for 3 to 5 min.
12. Wash the slide in running tap water (constant stream of water into container) for 10 min.
13. Place the slide in 70% alcohol plus ammonia for 3 min.
14. Place the slide in 95% alcohol for 5 min.
15. Place the slide in 100% alcohol for 5 min.
16. Place the slide in two changes of xylene for 5 min.

*This step can also be performed as follows.

- **A.** Place the slide in acid-alcohol decolorizer for 2 min.
- **B.** Wash the slide in running tap water (constant stream of water into container) for 1 min.
- **C.** Place the slide in acid-alcohol decolorizer for 2 min.
- **D.** Wash the slide in running tap water (constant stream of water into container) for 1 min.

REVIEW Permanent Stained Smears

Topic	Comments
Principle	To provide contrasting colors for the background debris and parasites present; designed to allow examination and recognition of detailed organism morphology under oil immersion examination (100x objective for a total magnification of ×1,000). Designed primarily to allow recovery and identification of the intestinal protozoa.
Specimen	Any stool specimen that is fresh or is preserved in fixative (mercury or non-mercury based with or without PVA), SAF, MIF, the newer single-vial-system fixatives, or Universal Fixative
Reagents	Trichrome, iron hematoxylin, modified iron hematoxylin, EcoStain, polychrome IV, or chlorazol black E stains and their associated solutions; dehydrating solutions (alcohols and xylenes); mounting fluid optional
Examination	Oil immersion examination of at least 300 fields; additional fields may be required if suspect organisms have been seen in the wet preparations from the concentrated specimen. A 50× or 60× oil immersion lens may be used for scanning; however, the final examination should be performed using the 100× oil immersion lens.
Results and Laboratory Reports	The majority of the suspect protozoa and/or human cells could be confirmed by the permanent stained smear. These reports should be categorized as "final" and would be signed out as such (the direct wet smear and concentration examination would provide "preliminary" results). However, if the testing turnaround time is 24 h, it is best to report the test results after the ova and parasite examination (fresh specimen, direct wet smear; concentration, permanent stained smear) is complete. Therefore, the reports would not be identified as "preliminary" or "final."
Procedure Notes and Limitations	The most commonly used stains include trichrome and iron hematoxylin. Unfortunately, helminth eggs and larvae take up too much stain and usually cannot be identified from the permanent stained smear. Also, coccidian oocysts and microsporidian spores usually require other special staining methods for identification. Permanent stained smears are normally examined under oil immersion examination (×1,000), and low or high dry power is not recommended. Confirmation of the intestinal protozoa (both trophozoites and cysts) is the primary purpose of this technique.

E. Continue the staining sequence with step 9 (iron hematoxylin working solution).

Procedure Notes for Modified Iron Hematoxylin Stain (Carbol Fuchsin Step)

1. The first 70% alcohol step acts with the Mayer's albumin to "glue" the specimen to the glass slide. The specimen may wash off if insufficient albumin is used or if the slides are too thick and are not completely dry before being stained.
2. The working hematoxylin stain should be checked each day of use by adding a drop of stain to alkaline tap water. If a blue color does not develop, prepare a fresh working stain solution.
3. The picric acid differentiates the hematoxylin stain by removing more stain from fecal debris than from the protozoa and removing more stain from the organism cytoplasm than from the nucleus. When properly stained, the background should be various shades of gray-blue and the protozoa, with medium blue cytoplasm and dark blue-black nuclei, should be easily seen.

Polychrome IV Stain

Polychrome IV stain can be used in place of trichrome for staining fecal smears by the MIF, PVA, or SAF fixative method. Both the stain and staining directions are available commercially (Devetec, Inc., P.O. Box 10275, Bradenton, FL 34282). Another source for the stain is Scientific Device Laboratory, Inc., P.O. Box 88, Glenview, IL 60025. Polychrome IV stain has been used primarily to stain permanent smears prepared from MIF-preserved fecal specimens.

Chlorazol Black E Stain

Chlorazol black E staining, developed by Kohn (9), is a method in which both fixation and staining occur in a single solution. This approach is used for fresh specimens, but it is not recommended for preserved material containing mercuric chloride (6) because it does not include an iodine-alcohol step, which is used to remove the mercuric chloride compound found in Schaudinn's fixative (with and without PVA) prepared with mercuric chloride. The optimal staining time must be determined for each batch of fixative-stain. The length of time for which the fixative-stain can be used depends on the number of slides run through the solution within a 30-day period. If the slides appear visibly red, the solution must be changed. This stain is not widely used.

Specialized Stains for Coccidia (*Cryptosporidium*, *Cystoisospora*, and *Cyclospora* Species) and the Microsporidia

Modified Kinyoun's Acid-Fast Stain (Cold Method)

Cryptosporidium spp. and *C. belli* have been recognized as causes of severe diarrhea in immunocompromised hosts but can also cause diarrhea in immunocompetent hosts (33). Oocysts in clinical specimens may be difficult to detect without special staining (34). Modified acid-fast stains are recommended to demonstrate these organisms. Unlike the Ziehl-Neelsen modified acid-fast stain, the modified Kinyoun's stain does not require the heating of reagents for staining (35). With additional reports of diarrheal outbreaks due to *Cyclospora* spp., it is also important to remember that these organisms are modified acid-fast variable and can be identified by this staining approach. Although the microsporidial spores are also acid fast, their size (1 to 2 μm) makes identification very difficult without special modified trichrome stains or the use of immunoassay reagents (36–41).

Concentrated (500 × *g* for 10 min) sediment of fresh, formalin-preserved, or other single-vial fixative-preserved stool may be used. Other types of clinical specimens such as duodenal fluid, bile, and pulmonary specimens (induced sputum, bronchial washings, or biopsy specimens) may also be stained (Fig. 3.22).

50% Ethanol

1. Add 50 ml of absolute ethanol to 50 ml of distilled water.
2. Store at room temperature. The solution is stable for 1 year. Note the expiration date on the label.

Kinyoun's Carbol Fuchsin

1. Dissolve 4 g of basic fuchsin in 20 ml of 95% ethanol (solution A).
2. Dissolve 8 g of phenol crystals in 100 ml of distilled water (solution B).
3. Mix solutions A and B.
4. Store at room temperature. The solution is stable for 1 year. Note the expiration date on the label.

1% Sulfuric Acid

1. Add 1 ml of concentrated sulfuric acid to 99 ml of distilled water.
2. Store at room temperature. The solution is stable for 1 year. Note the expiration date on the label.

Loeffler Alkaline Methylene Blue

1. Dissolve 0.3 g of methylene blue in 30 ml of 95% ethanol.

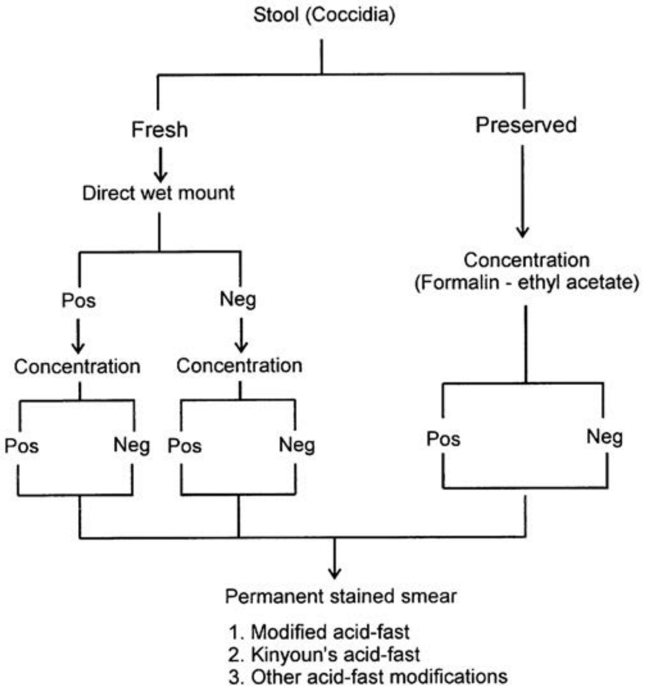

Stool (Coccidia)
Fresh
Preserved
Direct wet mount
Pos
Neg
Concentration
Concentration
Concentration
(Formalin - ethyl acetate)
Pos
Neg
Pos
Neg
Pos
Neg
Permanent stained smear
1. Modified acid-fast
2. Kinyoun's acid-fast
3. Other acid-fast modifications

Figure 3.22 Work flow diagram for fecal specimens (coccidia). The most important part of the procedure would be the concentrate (*Cytoisospora* sp.) and the permanent stained smear, using one of the modified acid-fast techniques (*Cryptosporidium* and *Cyclospora*). doi:10.1128/9781555819002.ch3.f22

2. Add 100 ml of dilute (0.01%) potassium hydroxide.
3. Store at room temperature. The solution is stable for 1 year. Note the expiration date on the label.

Quality Control for Kinyoun's Acid-Fast Stain

1. A control slide of *Cryptosporidium* from a 10% formalin-preserved specimen is included with each staining batch run. If the *Cryptosporidium* slide stains well, any *Cystoisospora* or *Cyclospora* oocysts present will also take up the stain, although *Cyclospora* oocysts tend to be acid-fast variable (Fig. 3.23).
2. *Cryptosporidium* spp. stain pink-red. Oocysts measure 4 to 6 µm, and four sporozoites may be visible within some oocysts. The background should stain uniformly blue.
3. The specimen is also checked for adherence to the slide (macroscopically).
4. The microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope). If the microscopes receive adequate maintenance and are not moved frequently, yearly recalibration may not be necessary.
5. Known positive microscope slides and photographs (reference books) should be available at the workstation.
6. Record all QC results; the laboratory should also have an action plan for "out-of-control" results.

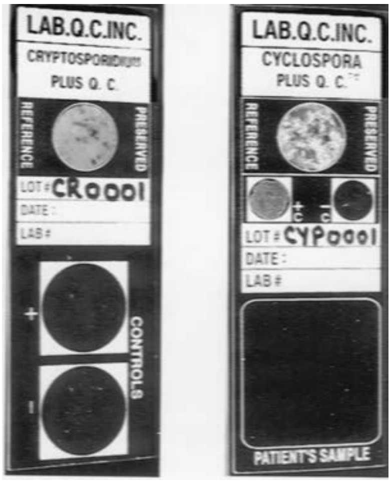

LAB.Q.C.INC.
CRYPTOSPORIDIUM
PLUS Q. C.
REFERENCE
PRESERVED
LOT # CR0001
DATE :
LAB #
+
-
CONTROLS
LAB.Q.C.INC.
CYCLOSPORA
PLUS Q. C.
REFERENCE
PRESERVED
LOT # CYP0001
DATE :
LAB #
PATIENT'S SAMPLE

Figure 3.23 Quality control slides for performing modified acid-fast stains (*Cryptosporidium* and *Cyclospora*) (Medical Chemical Corp.). doi:10.1128/9781555819002.ch3.f23

Procedure for Kinyoun's Acid-Fast Stain

1. Smear 1 to 2 drops of concentrated specimen sediment on the slide, and allow it to air dry. Do not make the smears too thick (you should be able to see through the wet material before it dries). Prepare two smears.
2. Fix with absolute methanol for 1 min.
3. Flood the slide with Kinyoun's carbol fuchsin and stain for 5 min.
4. Rinse the slide briefly (3 to 5 s) with 50% ethanol.
5. Rinse the slide thoroughly with water.
6. Decolorize with 1% sulfuric acid for 2 min or until no more color runs from the slide.
7. Rinse the slide with water. Drain.
8. Counterstain with methylene blue for 1 min.
9. Rinse the slide with water. Air dry.
10. Examine the slide with the low or high dry objective. To see the internal morphology, use an oil objective (100×).

Results and Patient Reports from Kinyoun's Acid-Fast Staining Method

The oocysts of *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* spp. stain pink to red to deep purple. Some of the

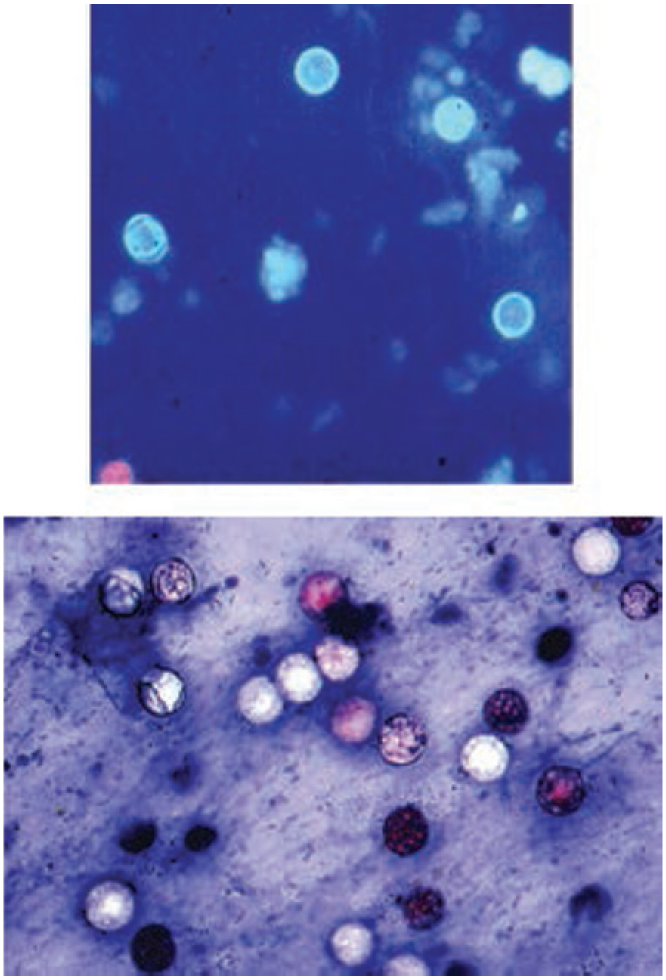


Figure 3.24 *Cyclospora cayetanensis* oocysts (8–10 µm). (Top) Autofluorescent oocysts; (bottom) oocysts stained with modified acid-fast stain. Note the range of color intensity; some oocysts resemble “wrinkled cellophane.” doi:10.1128/9781555819002.ch3.f24

four sporozoites may be visible in the *Cryptosporidium* oocysts. Some of the *Cystoisospora* immature oocysts (entire oocyst) will stain, while those that are mature usually appear with the two sporocysts within the oocyst wall stained pink to purple and a clear area between the stained sporocysts and the oocyst wall. The background stains blue. If *Cyclospora* oocysts are present (uncommon unless in an outbreak situation), they tend to be approximately 8 to 10 µm, they resemble *Cryptosporidium* spp. but are larger, and they have no definite internal morphology; the acid-fast staining tends to be more variable than that seen with *Cryptosporidium* or *Cystoisospora* spp. (Fig. 3.24 and 3.25). Some of the *Cyclospora* oocysts tend to look like “wrinkled cellophane,” with tremendous variation in color intensity ranging from clear to pink to red to deep purple to almost black. The stain intensity also depends on the thickness of the smear, the percentage of acid in the decolorizer (1% sulfuric acid recommended), and the length of time the smear is in contact with the decolorizer. If the patient has a heavy infection with microsporidia (immunocompromised patient), small (1- to 2-µm) spores may be seen but may not be recognized as anything other than bacteria or small yeast cells.

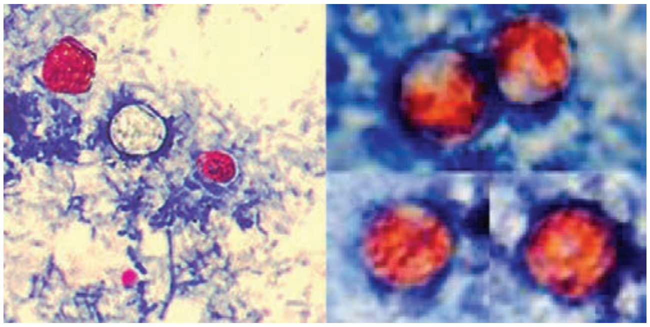
There is usually a range of color intensity in the organisms present; not every oocyst appears deep pink to purple. The greatest staining variation will be seen with *Cyclospora* oocysts; do not decolorize too long.

1. Report the organism and stage (oocyst). Do not use abbreviations.
   Examples: *Cryptosporidium* spp. oocysts
   Cystoisospora belli oocysts
2. Call the physician when these organisms are identified.
3. Save positive slides for future reference. Label prior to storage (name, patient number, organisms present).

Procedure Notes for Kinyoun’s Acid-Fast Staining Method

1. Routine stool examination stains (trichrome and iron hematoxylin) are not recommended; however, sedimentation concentration (500 × *g* for 10 min) is recommended for the recovery and identification of the coccidia after the concentration sediment has been stained with one of the modified acid-fast stains. The routine concentration (formalin-ethyl

Figure 3.25 (Left) *Cyclospora* (8 to 10 µm), *Cryptosporidium* (4 to 6 µm), and artifact (~2 µm) stained with modified acid-fast stain. Note that one of the *Cyclospora* oocysts did not stain (modified acid-fast variable) and has the “wrinkled-cellophane” appearance, while the other oocyst displays the typical purple/red color; the *Cryptosporidium* oocyst and the artifact did stain modified acid-fast positive. These artifacts are frequently seen; it is very important to measure objects seen in the modified acid-fast smears to confirm that they are actual parasites and not artifacts. (Right) *Cyclospora* stained with a hot safranin stain (higher magnification than the image on the left). (Courtesy of CDC.) doi:10.1128/9781555819002.ch3.f25



acetate) can be used to recover *Cystoisospora* oocysts (wet sediment examination and/or modified acid-fast stains), but routine permanent stains (trichrome and iron hematoxylin) are not reliable for this purpose.

2. Preserved specimens containing PVA are not acceptable for staining with the modified acid-fast stain. However, specimens preserved in SAF, other non-mercury single-vial-system fixatives, or those preserved in Universal Fixative are perfectly acceptable.
3. Avoid the use of wet-gauze filtration (an old, standardized method of filtering stool prior to centrifugation) with too many layers of gauze that may trap organisms and not allow them to flow into the fluid to be concentrated. It is recommended that no more than two layers of gauze be used; **another option is to use the commercially available concentrators that use plastic or metal screens instead of gauze.**
4. Other organisms that stain positive include acid-fast bacteria, *Nocardia* spp., and the microsporidia (which are very difficult to find and identify even when they appear to be acid fast).
5. It is very important that smears not be too thick. Thicker smears may not adequately destain.
6. Concentration of the specimen is essential to demonstrate organisms. The number of organisms seen in the specimen may vary from many to very few; therefore, the organisms are concentrated in the sediment, which is used to prepare the smears for subsequent staining.
7. Some specimens require treatment with 10% KOH because of their mucoid consistency. Add 10 drops of 10% KOH to the sediment, and vortex until homogeneous. Rinse with 10% formalin, and centrifuge (500 × *g* for 10 min). Without decanting the supernatant, take 1 drop of the sediment and smear it thinly on a slide.
8. Commercial concentrators and reagents are available. (See Appendix 1.)
9. Concentrations of sulfuric acid of 1.0 to 3.0% are normally used. Higher concentrations remove too much stain. The use of acid-alcohol (routinely used in the Ziehl-Neelsen acid-fast staining method for the mycobacteria) decolorizes all organisms; therefore, one must use the modified decolorizer (1 to 3% H_2SO_4) for good results. In general, 1% acid is recommended; this approach will provide excellent staining results for all the coccidia.
10. There is some debate whether organisms lose their ability to take up the acid-fast stain after long-term storage in 10% formalin. Some laboratories have reported this diminished staining.
11. Specimens should be centrifuged in capped tubes, and gloves should be worn during all phases of specimen processing.

Procedure Limitations for Kinyoun's Acid-Fast Staining Method

1. Light infections with *Cryptosporidium* spp. may be missed (small number of oocysts). The fecal immunoassay methods are more sensitive.
2. Multiple specimens must be examined, since the numbers of oocysts present in the stool vary from day to day. A series of three specimens submitted on alternate days is recommended.
3. *Cyclospora* may be suspected if the organisms appear to be *Cryptosporidium* but are about twice the size (about 10 μm) (Fig. 3.25). The microsporidial spores are extremely small (1 to 2 μm) and will probably not be recognized unless they are very numerous and appear to have a somewhat different morphology from the bacteria in the preparation.

Modified Ziehl-Neelsen Acid-Fast Stain (Hot Method)

Cryptosporidium and *Cystoisospora* have been recognized as causes of severe diarrhea in immunocompromised hosts but can also cause diarrhea in immunocompetent hosts. Oocysts in clinical specimens may be difficult to detect without special staining. Modified acid-fast stains are recommended to demonstrate these organisms. Application of heat to the carbol fuchsin assists in the staining, and the use of a milder decolorizer allows the organisms to retain their pink-red color (37). With continued reports of diarrheal outbreaks due to *Cyclospora*, it is also important to remember that these organisms are acid fast and can be identified by using this staining approach (42, 43). Although the microsporidial spores are also acid fast, their size (1 to 2 μm) makes identification very difficult without special stains or the use of monoclonal antibody reagents.

Concentrated sediment of fresh, formalin, other non-mercury single-vial fixative-preserved stool, or those preserved in Universal Fixative may be used. Other types of clinical specimens such as duodenal fluid, bile, and pulmonary specimens (induced sputum, bronchial washings, or biopsy specimens) may also be stained.

Carbol Fuchsin

1. To make basic fuchsin (solution A), dissolve 0.3 g of basic fuchsin in 10 ml of 95% ethanol.
2. To make phenol (solution B), dissolve 5 g of phenol crystals in 100 ml of distilled water. (Gentle heat may be needed.)

3. Mix solution A with solution B.
4. Store at room temperature. The solution is stable for 1 year. Note the expiration date on the label.

1–3% Sulfuric Acid

1. Add 1 to 3 ml of concentrated sulfuric acid to 99 or 97 ml of distilled water.
2. Store at room temperature. The solution is stable for 1 year. Note the expiration date on the label.

Methylene Blue

1. Dissolve 0.3 g of methylene blue chloride in 100 ml of distilled water.
2. Store at room temperature. The solution is stable for 1 year. Note the expiration date on the label.

Quality Control for the Modified Ziehl-Neelsen Acid-Fast Staining Method

QC guidelines are the same as those for the Kinyoun's acid-fast stain and are given on p. 53.

Procedure for the Modified Ziehl-Neelsen Staining Method

1. Smear 1 to 2 drops of specimen on the slide, and allow it to air dry. Do not make the smears too thick (you should be able to see through the wet material before it dries). Prepare two smears.
2. Dry on a heating block (70°C) for 5 min.
3. Place the slide on a staining rack, and flood it with carbol fuchsin.
4. With an alcohol lamp or Bunsen burner, gently heat the slide to steaming by passing a flame under the slide. Discontinue heating once the stain begins to steam. Do not boil.
5. Allow the specimen to stain for 5 min. If the slide dries, add more stain without additional heating.
6. Rinse thoroughly with water. Drain.
7. Decolorize with 1–3% sulfuric acid for 30 s. (Thicker slides may require a longer destain.)
8. Rinse the slide with water. Drain.
9. Flood the slide with methylene blue for 1 min.
10. Rinse the slide with water, drain, and air dry.
11. Examine with low or high dry objective. To see internal morphology, use the oil objective (100×).

Results and Patient Reports from the Modified Ziehl-Neelsen Acid-Fast Staining Method

The oocysts of *Cryptosporidium* and *Cystoisospora* spp. stain pink to red to deep purple. Some of the four sporozoites may be visible in the *Cryptosporidium* oocysts. Some of the *Cystoisospora* immature oocysts (entire oocyst) stain, while those that are mature usually appear with the two sporocysts within the oocyst wall stained a pink to purple color and a clear area between the stained sporocysts and the oocyst wall (Fig. 3.26). The background stains blue. If *Cyclospora* oocysts are present (uncommon), they tend to be approximately 8 to 10 µm, they resemble *Cryptosporidium* oocysts but are larger, and they have no definite internal morphology; the acid-fast staining tends to be more variable than that seen with *Cryptosporidium* or *Cystoisospora* spp. If the patient has a heavy infection with microsporidia (immunocompromised patient), small (1- to 2-µm) spores may be seen but may not be recognized as anything other than bacteria or small yeast cells.

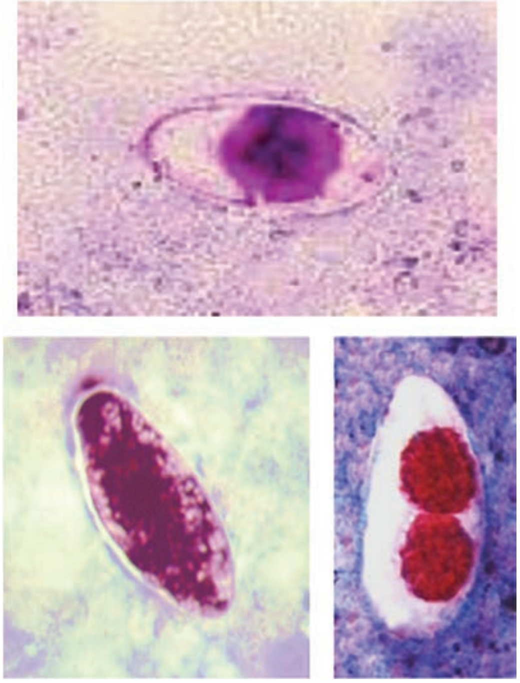


Figure 3.26 (Upper) Immature *Cystoisospora belli* oocyst stained with modified acid-fast stain. (Lower) *C. belli* immature oocyst (left) (note that the entire oocyst retains the stain) and *C. belli* mature oocyst containing two sporocysts (right). doi:10.1128/9781555819002.ch3.f26

There is usually a range of color intensity in the organisms present; not every oocyst appears deep pink to purple. The greatest staining variation would be seen with *Cyclospora* organisms.

1. Report the organism and stage (oocyst). Do not use abbreviations.
   Examples: *Cryptosporidium* spp. oocysts
   Cystoisospora belli oocysts
   Cyclospora cayetanensis oocysts
2. Call the physician when these organisms are identified.
3. Save positive slides for future reference. Label prior to storage (name, patient number, organisms present).

Procedure Notes for the Modified Ziehl-Neelsen Acid-Fast Staining Method

1. Routine stool examination stains (trichrome and iron hematoxylin) are not recommended; however, sedimentation concentration (500 × *g* for 10 min) is acceptable for the recovery and identification of the coccidia, particularly after the concentration sediment has been stained with one of the modified acid-fast stains. The routine concentration (formalin-ethyl acetate) can be used to recover *Cystoisospora* oocysts (wet sediment examination and/or modified acid-fast stains), but routine permanent stains (trichrome and iron hematoxylin) are not reliable for this purpose.
2. Preserved specimens containing PVA are not acceptable for staining with the modified acid-fast stain. However, specimens preserved in SAF, other single-vial preservatives, or the Universal Fixative are perfectly acceptable.
3. Avoid the use of wet-gauze filtration (an old, standardized method of filtering stool prior to centrifugation) with too many layers of gauze that may trap organisms and not allow them to flow into the fluid to be concentrated. It is recommended that no more than two layers of gauze be used. Another option is to use the commercially available concentration systems in which metal or plastic screens are used for filtration.
4. Other organisms that stain positive include acid-fast bacteria, *Nocardia* spp., and the microsporidia (which are very difficult to find and identify even when they appear to be acid fast).
5. It is very important that smears not be too thick. Thicker smears may not adequately destain.
6. Concentration of the specimen is essential to demonstrate organisms. The number of organisms seen in the specimen may vary from numerous to very few. Therefore, the sensitivity of the method is enhanced if the stains are performed on concentrated sediment.
7. Some specimens require treatment with 10% KOH because of their mucoid consistency. Add 10 drops

REVIEW Modified Acid-Fast Smears

Topic	Comments
Principle	To provide contrasting colors for both the background debris and parasites present; designed to allow examination and recognition of acid-fast characteristic of the organisms under high dry examination (40× objective for a total magnification of ×400). Designed primarily to allow recovery and identification of intestinal coccidian oocysts. Internal morphology (sporozoites) is seen in some *Cryptosporidium* oocysts under oil immersion (×1,000 magnification).
Specimen	Any stool specimen that is fresh or preserved in formalin, SAF, or the newer single-vial-system fixatives
Reagents	Kinyoun's acid-fast stain, modified Ziehl-Neelsen stain, and their associated solutions; dehydrating solutions (alcohols and xylenes); mounting fluid optional; the decolorizing agents are less intense than the routine acid-alcohol used in routine acid-fast staining (this fact is what makes these procedures "modified" acid-fast procedures).
Examination	High dry examination of at least 300 fields; additional fields may be required if suspect organisms have been seen but are not clearly acid fast.
Results and Laboratory Reports	The identification of *Cryptosporidium* and *Cystoisospora* oocysts should be possible; *Cyclospora* oocysts, which are twice the size of *Cryptosporidium* oocysts, should be visible but tend to be more acid-fast variable. The stain intensity depends on the thickness of the smear, the percentage of acid in the decolorizer, and the length of time the smear is in contact with the decolorizer. Although microsporidia are acid fast, their small size makes their recognition very difficult. Final laboratory results would depend heavily on the appearance of the QC slides and comparison with patient specimens.
Procedure Notes and Limitations	Both the cold and hot modified acid-fast methods are excellent for the staining of coccidial oocysts. There is some feeling that the hot method may result in better stain penetration, but the differences are probably minimal. Procedure limitations are related to specimen handling (proper centrifugation speeds and time, use of no more than two layers of wet gauze for filtration, and complete understanding of the difficulties in recognizing microsporidial spores). There is also some controversy concerning whether the organisms lose the ability to take up acid-fast stains after long-term storage in 10% formalin. The organisms are more difficult to find in specimens from patients who do not have the typical, watery diarrhea (more formed stool contains more artifact material).

of 10% KOH to the sediment, and vortex until homogeneous. Rinse with 10% formalin, and centrifuge (500 × *g* for 10 min). Without decanting the supernatant, take 1 drop of the sediment and smear it thinly on a slide.

8. Commercial concentrators and reagents are available.
9. Do not boil the stain. Gently heat until steam rises from the slide. Do not allow the stain to dry on the slide.
10. Various concentrations of sulfuric acid (0.25 to 10%) may be used; the destaining time varies according to the concentration used. Generally, a 1 or 3% solution is used. The use of acid-alcohol (routinely used in the Ziehl-Neelsen acid-fast staining method for the mycobacteria) decolorizes all organisms; therefore, the modified decolorizer must be used for good results.
11. There is some debate whether organisms lose their ability to take up the acid-fast stain after long-term storage in 10% formalin. Some laboratories have reported this diminished staining.
12. Specimens should be centrifuged in capped tubes, and gloves should be worn during all phases of specimen processing.

Procedure Limitations for the Modified Ziehl-Neelsen Acid-Fast Staining Method

1. Light infections with *Cryptosporidium* or *Cyclospora* may be missed (small number of oocysts). When available, fecal immunoassay methods for *Cryptosporidium* are more sensitive.
2. Multiple specimens must be examined, since the numbers of oocysts present in the stool vary from day to day. A series of three specimens submitted on alternate days is recommended.
3. The identification of both *Cyclospora* organisms and microsporidia may be difficult. *Cyclospora* may be suspected if the organisms appear to be *Cryptosporidium* but are about twice the size (about 8 to 10 µm). The microsporidial spores are extremely small (1 to 2 µm) and will probably not be recognized unless they are very numerous and appear to have a somewhat different morphology from the bacteria in the preparation.
4. Often, artifact material may be seen in these stained smears (Fig. 3.25). The artifacts may resemble the oocysts of *Cryptosporidium* or *Cyclospora*; therefore, it is very important that any "parasites" seen in the stained smears be measured for confirmation.

There are three other stains that can be used for the coccidia, although they may not be as common as the Kinyoun or hot acid-fast method. They are the carbol fuchsin negative stain, the safranin stain, and the auramine O stain.

Carbol Fuchsin Negative Stain for *Cryptosporidium* (from W. L. Current)

1. Mix thoroughly an equal volume (3 to 10 µl) of fresh or formalin-fixed stool and Kinyoun's carbol fuchsin on a slide.
2. Spread out as a thin film, and allow to air dry at room temperature.
3. Add immersion oil directly to the stained smear, and then cover with a coverslip.
4. Observe under bright-field microscopy (×400). Everything but the oocysts stains darkly. The oocysts are bright and refractile because they contain water and everything else is oil soluble.

Rapid Safranin Method for *Cryptosporidium* (36)

1. Smear fresh or formalin-fixed feces on a slide, and allow the film to air dry at room temperature.
2. Fix briefly with one pass through the Bunsen burner flame.
3. Fix for 3 to 5 min with 3% HCl in methanol.
4. Wash with tap water (brief rinse).
5. Stain with 1% aqueous safranin for 1 min (heat until steam appears) (the authors indicate by personal communication that boiling may be beneficial).
6. Rinse in tap water.
7. Counterstain with 1% methylene blue for 30 s (0.1% aqueous crystal violet was almost as good, but malachite green was unsatisfactory).

Rapid Safranin Method for *Cyclospora*, Using a Microwave Oven (40, 44)

Another rapid safranin method uniformly stains *Cyclospora* oocysts a brilliant reddish orange. However, the fecal smears must be heated in a microwave oven before being stained. This stain is fast, reliable, and easy to perform (40) (Fig. 3.25).

1. Using a 10-µl aliquot of concentrated stool, prepare the smear by spreading the material thinly across the slide.
2. Allow the smear to dry on a 60°C slide warmer.
3. Cool the slide to room temperature before staining.
4. Place the slide in a Coplin jar containing acidic alcohol (3% [vol/vol] HCl in methanol), and let it stand for 5 min.

5. Wash off excess acidic alcohol with cold tap water.
6. Place the slide(s) into the Coplin jar containing the safranin solution in acidified water (pH 6.5), and microwave at full power (650 W) for 1 min. (Place the staining jar in another container to catch the overflow of stain because of boiling.)
7. Wash off excess stain with cold tap water.
8. Place the slide(s) for 1 min in a Coplin jar containing 1% methylene blue.
9. Rinse gently with cold tap water.
10. Air dry.
11. Add a coverslip to the slide by using Cytoseal 60 or other mounting medium; the immersion oil mounting method can also be used.
12. Examine the smear with low-power or high dry power objectives. To see additional morphology, use the oil immersion objective (100×).

Auramine O Stain for Coccidia (from Thomas Hänscheid)

Coccidia are acid-fast organisms and also stain well with auramine O (phenolized auramine O). The size and typical appearance of *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* oocysts enable auramine O-stained slides to be examined at low power using the 10× objective. The entire sample area can usually be examined in less than 30 s. The low cost of the reagents, the simple staining protocol, and the rapid microscopic examination also make this staining method suitable for screening unconcentrated fecal specimens.

Concentrated sediment from fresh or non-PVA-preserved stool may be used. Other stool samples may also be used, such as unconcentrated stool submitted for culture in a bacteriology transport medium. However, to increase the sensitivity of the test, small numbers of oocysts are more easily detected using concentrated stools.

Auramine O Stain

Solution 1

Auramine O	0.1 g
95% ethanol	10 ml

Solution 2

Phenol crystals	3.0 g
Distilled water	87 ml

Combine solutions 1 and 2. Store in a dark bottle at room temperature for up to 3 months.

Destaining Agent (0.5% Acid-Alcohol)

Concentrated HCl	0.5 ml
70% ethanol	100 ml

Store at room temperature for up to 3 months.

Counterstain (0.5% Potassium Permanganate)

Potassium permanganate	0.5 g
Distilled water	100 ml

Quality Control for the Auramine O Stain for Coccidia

QC guidelines are the same as those for the Kinyoun's acid-fast stain and are given on p. 53.

Procedure for the Auramine O Stain for Coccidia

1. Using a 10- to 20-µl aliquot of concentrated stool, prepare the smear by spreading the material across the slide.
2. Heat fix the slides either on a 65 to 75°C heat block for at least 2 h or with the flame of a Bunsen burner. However, do not overheat. Another fixation option would be to fix the slide in absolute methanol for 1 min, air dry, and then proceed with staining.
3. Cool the slide to room temperature before staining.
4. Flood the slide with the phenolized auramine O solution.
5. Allow the smear to stain for ca. 15 min. *Do not heat.*
6. Rinse the slide in water. Drain excess water from the slide.
7. Flood the slide with the destaining solution (0.5% acid-alcohol).
8. Allow to decolorize for 2 min.
9. Flood with counterstain (potassium permanganate) solution.
10. Stain for 2 min. *The timing of this step is critical.*
11. Rinse the slide in water. Drain excess water from the slide.
12. Allow the smear to air dry. Do not blot.
13. Examine the smear with a fluorescence microscope with a 10× objective and fluorescein isothiocyanate optical filters (auramine O: excitation max., ~435 nm in water; emission max., ~510 nm). Screen the whole sample area for the presence of fluorescent oocysts. Suspicious objects can be reexamined using a 20× or 100× objective.
14. Smears can be restained by any of the carbol fuchsin (modified acid-fast) staining procedures to allow examination with light microscopy.

Results and Patient Reports from the Auramine O Staining Method

Cryptosporidium and *Cyclospora* oocysts fluoresce brightly and have a regular round appearance ("starry sky" appearance with the 10× objective). In contrast to the large majority of fluorescent artifacts, the oocysts do not stain homogeneously. Thus, the fluorescence is heterogeneously distributed in the interior of the oocyst (Fig. 3.27). *Cystoisospora* oocysts fluoresce brightly with three patterns: (i) a more or less brightly but heterogeneously stained interior of the whole oocyst, (ii) one brightly staining sporocyst, or (iii) two brightly staining sporocysts within the oocyst wall.

1. Report the organism and stage (oocyst). Do not use abbreviations.
   Examples: *Cryptosporidium* spp. oocysts
   Cystoisospora belli oocysts
   Cyclospora cayetanensis oocysts
2. Call the physician when these organisms are identified.
3. Save positive slides for future reference. Label prior to storage (name, patient number, organisms present). These slides can be kept at room temperature in the dark, and the fluorescence remains stable for up to 3 to 4 weeks.

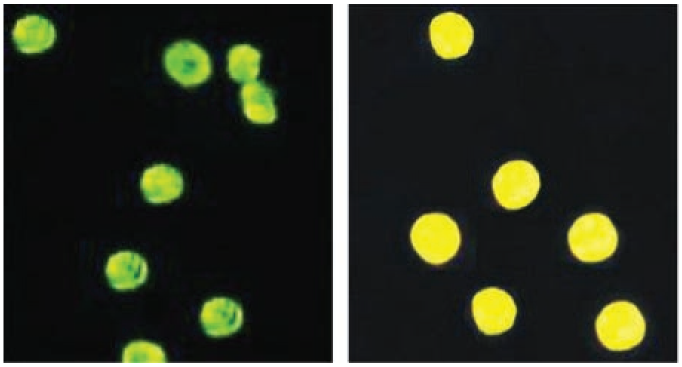
Procedure Notes for the Auramine O Staining Method

1. It is mandatory that positive control smears be stained and examined each time patient specimens are stained and examined.
2. For best results, examine the auramine O solution for deposits and remove them by filtration or centrifugation. This problem can also be avoided by preparing smaller volumes more frequently.
3. Slides should be observed as soon as possible after staining. However, they can be kept at room temperature in the dark, and fluorescence remains stable for up to 3 to 4 weeks.

Procedure Limitations for the Auramine O Staining Method

1. Light infections might be missed, particularly if unconcentrated stool is used; it is always recommended that concentrated stool sediment be used for staining.
2. Use of the 40× high dry objective often causes a blurred image (fluorescent "halo" around the image, hazy contours), which appears to be the effect of interfering fluorescence from the auramine O stain located outside the plane of focus (Fig. 3.27). The 100× oil immersion objective gives better-quality images. Immersion oils used for light microscopy may be autofluorescent, and special low-fluorescence immersion oil should be used.
3. If the fluorescence is not clear or definitive, a suspicious slide can be restained with a modified acid-fast stain and reexamined using light microscopy and the 100× oil immersion objective.
4. If protected from sunlight, auramine O slides can be kept on the bench at room temperature for up to 2 to 3 weeks, with only minor loss of fluorescence (photo bleaching).

Figure 3.27 *Cryptosporidium* spp. oocysts stained with auramine O fluorescent stain (using different filters). doi:10.1128/9781555819002.ch3.f27



Modified Trichrome Stain for the Microsporidia (Weber—Green) (41)

The diagnosis of intestinal microsporidiosis (*Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*) has depended on the use of invasive procedures and subsequent examination of biopsy specimens, often by electron microscopy. However, the need for a practical method for the routine clinical laboratory has stimulated some work in the development of additional methods. Slides prepared from fresh, formalin-fixed, or stools preserved in the Universal Fixative can be stained by a chromotrope-based technique and can be examined under light microscopy. This staining method is based on the fact that stain penetration of the microsporidial spore is very difficult; therefore, the dye content in the chromotrope 2R is greater than that routinely used to prepare Wheatley's modification of Gomori's trichrome method, and the staining time is much longer (90 min) (Fig. 3.28 and 3.29) (39, 41). At least several of these stains are available commercially from a number of suppliers.

The specimen can be fresh stool or stool that has been preserved in 5 or 10% formalin, SAF, or the newer single-vial-system fixatives (Universal Fixative—no PVA). Actually, any specimen other than tissue thought to contain microsporidia could be stained by this method.

Trichrome Stain (Modified for Microsporidia) (Weber—Green) (41)

Chromotrope 2R	6.0 g*
Fast green	0.15 g
Phosphotungstic acid	0.7 g
Acetic acid (glacial)	3.0 ml
Distilled water	100.0 ml

*(10 times the normal trichrome stain formula)

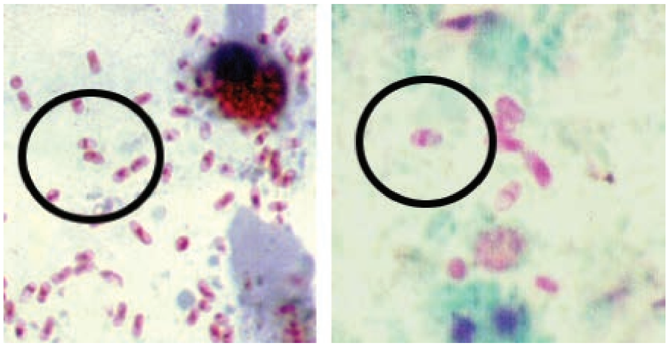


Figure 3.28 (Left) Microsporidian spores in a nasopharyngeal specimen stained with a Ryan modified trichrome stain; (right) microsporidian spores in stool stained with a Weber modified trichrome stain. The spores range from about 1.5 to 2.0 µm in diameter. Note the horizontal lines, indicating the presence of a polar tubule. doi:10.1128/9781555819002.ch3.f28

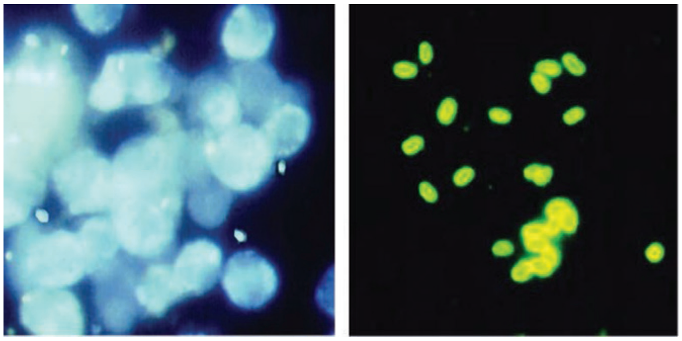
1. Prepare the stain by adding 3.0 ml of acetic acid to the dry ingredients. Allow the mixture to stand (ripen) for 30 min at room temperature.
2. Add 100 ml of distilled water. Properly prepared stain is dark purple.
3. Store in a glass or plastic bottle at room temperature. The shelf life is at least 24 months.

Acid-Alcohol

90% ethyl alcohol	995.5 ml
Acetic acid (glacial)	4.5 ml

Prepare by combining the two solutions.

Figure 3.29 (Left) Microsporidian spores in a urine sediment, stained with calcofluor white stain; (right) *Encephalitozoon intestinalis* spores stained with an organism-specific fluorescent antibody reagent. Note that some of the spores are intracellular. A urine specimen tends to be "cleaner" than stool; therefore the spores may be easier to see and identify in urine or any specimen that contains less artifact material than stool. doi:10.1128/9781555819002.ch3.f29



Quality Control for the Modified Trichrome Staining Method (Weber—Green)

1. Unfortunately, the only way to perform acceptable QC procedures for the modified trichrome staining method is to use actual microsporidial spores as the control organisms. Obtaining these positive controls may be somewhat difficult. It is particularly important to use the actual organisms, because the spores are difficult to stain and they are very small (1 to 2.5 µm).
2. A QC slide should be included with each run of stained slides, particularly if the staining setup is used infrequently.
3. All staining dishes should be covered to prevent evaporation of reagents (screw-cap Coplin jars or glass lids).
4. Depending on the volume of slides stained, staining solutions must be changed on an as-needed basis.
5. When the smear is thoroughly fixed and the stain is performed correctly, the spores are ovoid and refractile, with the spore wall being bright pinkish red. Occasionally, the polar tube can be seen either as a stripe or as a diagonal line across the spore. The majority of the bacteria and other debris tend to stain green. However, some bacteria and debris still stain red.
6. The specimen is also checked for adherence to the slide (macroscopically).
7. The microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope). Although recalibration every 12 months may not be necessary, this will vary from laboratory to laboratory, depending on equipment care and use.
8. Known positive microscope slides and photographs (reference books) should be available at the workstation.
9. Record all QC results; the laboratory should also have an action plan for "out-of-control" results.

Procedure for Modified Trichrome Staining Method (Weber—Green)

1. Using a 10-µl aliquot of unconcentrated (concentrated recommended), preserved liquid stool (5 or 10% formalin or SAF or one of the non-PVA single-vial preservatives), prepare the smear by spreading the material over an area 45 by 25 mm.
2. Allow the smear to air dry.
3. Place the smear in absolute methanol for 5 min.
4. Allow the smear to air dry.

5. Place in trichrome stain for 90 min.
6. Rinse in acid-alcohol for no more than 10 s.
7. Dip slides several times in 95% alcohol. Use this step as a rinse.
8. Place in 95% alcohol for 5 min.
9. Place in 100% alcohol for 10 min.
10. Place in xylene substitute for 10 min.
11. Mount with a coverslip (no. 1 thickness), using mounting medium.
12. Examine smears under oil immersion (×1,000), and read at least 300 fields; the examination time will probably be at least 10 min per slide.

Modified Trichrome Stain for the Microsporidia (Ryan—Blue) (39)

A number of variations to the modified trichrome stain (Weber—green) have been tried in an attempt to improve the contrast between the color of the spores and the background staining. Optimal staining was achieved by modifying the composition of the trichrome solution. This stain is also available commercially from a number of suppliers.

The specimen can be fresh stool or stool that has been preserved in 5 or 10% formalin, SAF, or the newer single-vial-system fixatives. Actually, any specimen other than tissue thought to contain microsporidia could be stained by this method.

Trichrome Stain (Modified for Microsporidia) (Ryan—Blue)

Chromotrope 2R	6.0 g*
Aniline blue	0.5 g
Phosphotungstic acid	0.25 g
Acetic acid (glacial)	3.0 ml
Distilled water	100.0 ml

*(10 times the normal trichrome stain formula)

1. Prepare the stain by adding 3.0 ml of acetic acid to the dry ingredients. Allow the mixture to stand (ripen) for 30 min at room temperature.
2. Add 100 ml of distilled water, and adjust the pH to 2.5 with 1.0 M HCl. Properly prepared stain is dark purple. The staining solution should be protected from light.
3. Store in a glass or plastic bottle at room temperature. The shelf life is at least 24 months.

Acid-Alcohol

90% ethyl alcohol	995.5 ml
Acetic acid (glacial)	4.5 ml

Prepare by combining the two solutions.

Quality Control for the Modified Trichrome Staining Method (Ryan—Blue)

1. Unfortunately, the only way to perform acceptable QC procedures for this method is to use actual microsporidial spores as the control organisms. Obtaining these positive controls may be somewhat difficult. It is particularly important to use the actual organisms, because the spores are difficult to stain and are very small (1 to 2.5 µm).
2. A QC slide should be included with each run of stained slides, particularly if the staining setup is used infrequently.
3. All staining dishes should be covered to prevent evaporation of reagents (screw-cap Coplin jars or glass lids).
4. Depending on the volume of slides stained, staining solutions must be changed on an as-needed basis.
5. When the smear is thoroughly fixed and the stain is performed correctly, the spores are ovoid and refractile, with the spore wall being bright pinkish red. Occasionally, the polar tube can be seen either as a stripe or as a diagonal line across the spore. The majority of the bacteria and other debris tend to stain blue. However, some bacteria and debris still stain red.
6. The specimen is also checked for adherence to the slide (macroscopically).
7. The microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope). Although recalibration every 12 months may not be necessary, this will vary from laboratory to laboratory, depending on equipment care and use.
8. Known positive microscope slides and photographs (reference books) should be available at the workstation.
9. Record all QC results; the laboratory should also have an action plan for "out-of-control" results.

Procedure for the Modified Trichrome Staining Method (Ryan—Blue)

1. Using a 10-µl aliquot of concentrated (10 min at 500 × *g*), preserved liquid stool (5 or 10% formalin, SAF, one of the non-PVA single-vial preservatives, zinc based, or Universal Fixative), prepare the smear by spreading the material over an area 45 by 25 mm.
2. Allow the smear to air dry.
3. Place the smear in absolute methanol for 5 or 10 min.
4. Allow the smear to air dry.
5. Place in trichrome stain for 90 min.
6. Rinse in acid-alcohol for no more than 10 s.

7. Dip the slides several times in 95% alcohol. Use this step as a rinse (no more than 10 s).
8. Place in 95% alcohol for 5 min.
9. Place in 95% alcohol for 5 min.
10. Place in 100% alcohol for 10 min.
11. Place in xylene substitute for 10 min.
12. Mount with a coverslip (no. 1 thickness), using mounting medium.
13. Examine smears under oil immersion (×1,000), and read at least 300 fields; the examination time will probably be at least 10 min per slide.

Results and Patient Reports from Modified Trichrome Staining Methods (Weber or Ryan)

The microsporidial spore wall should stain pinkish to red, with the interior of the spore being clear or perhaps showing a horizontal or diagonal stripe which represents the polar tube. The background appears green or blue, depending on the method. Bacteria, some yeast cells, and some debris stain pink to red; the shapes and sizes of the various components may be helpful in differentiating the spores from other structures. The results of this staining procedure should be reported only if the positive control smears are acceptable. The production of immunoassay reagents should provide a more specific and sensitive approach to the identification of the microsporidia in fecal specimens.

1. Report the organism.
   Examples: Microsporidial spores present. The following information can be added to the report to assist the physician in treating and following the patient:
   The organisms are most probably *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis* (if from fecal specimen or urine).
   The organisms are most probably *Encephalitozoon intestinalis* (identification to species highly likely) (generally the organism involved in disseminated cases from the gastrointestinal tract to other body sites).

Procedure Notes for Modified Trichrome Staining Methods (Weber or Ryan)

1. It is mandatory that positive control smears be stained and examined each time patient specimens are stained and examined.
2. Because of the difficulty in getting the stain to penetrate the spore wall, prepare thin smears and do not reduce the staining time in trichrome. Also, make sure the slides are not left too long in the decolorizing agent (acid-alcohol). If the control organisms are too light, leave them in the trichrome longer and shorten the time to two dips in the acid-alcohol solution. Also, remember that the 95% alcohol rinse after the acid-alcohol should be performed quickly to prevent additional destaining from the acid-alcohol reagent.
3. When you purchase the chromotrope 2R, obtain the highest dye content available. Two sources are Harleco, Gibbstown, NJ, and Sigma Chemical Co., St. Louis, MO (dye content among the highest [85%]). Fast green and aniline blue can be obtained from Allied Chemical and Dye, New York, NY. See also appendix 5.
4. In the final stages of dehydration, the 100% ethanol and the xylenes (or xylene substitutes) should be kept as free from water as possible. Coplin jars must have tight-fitting caps to prevent both evaporation of reagents and absorption of moisture. If the xylene or xylene substitute becomes cloudy after addition of slides from 100% alcohol, return the slides to fresh 100% alcohol and also replace the xylene or xylene substitute with fresh stock.

Procedure Limitations for Modified Trichrome Staining Methods (Weber or Ryan)

1. Although this staining method stains the microsporidia, the range of stain intensity and the small size of the spores cause some difficulty in identifying these organisms. Since this procedure results in many other organisms or objects staining in stool specimens, differentiation of the microsporidia from surrounding material is still very difficult. There also tends to be some slight size variation among the spores.
2. If the patient has severe watery diarrhea, there is less artifact material in the stool to confuse with the microsporidial spores; however, if the stool is semiformed or formed, the amount of artifact material is much greater and the spores are much harder to detect and identify. Also, the number of spores varies according to the stool consistency (generally, the more liquid the stool, the more spores will be present). However, remember that there is also a dilution factor if the stool is very liquid.
3. The workers who developed some of these procedures think that concentration procedures result in an actual loss of microsporidial spores; therefore, there is a recommendation to use unconcentrated, formalinized stool. **However, there are no data indicating which centrifugation speeds, etc., were used in the study.**
4. In the UCLA Clinical Microbiology Laboratory, we have generated data (unpublished) to indicate that **centrifugation at 500 × *g* for 10 min dramatically increases the number of microsporidial spores available for staining (from the concentrate sedi-**

REVIEW Modified Trichrome-Stained Smears	
Topic	**Comments**
Principle	To provide contrasting colors for the background debris and parasites present; designed to allow examination and recognition of organism morphology under oil immersion (100× objective for a total magnification of ×1,000). Designed primarily to allow recovery and identification of microsporidial spores. Internal morphology (horizontal or diagonal "stripes") may be seen in some spores under oil immersion (×1,000 magnification).
Specimen	Any stool specimen that is fresh or is preserved in formalin, SAF, one of the single-vial-system fixatives, or the Universal Fixative
Reagents	Modified trichrome stain (using high-dye-content chromotrope 2R) and associated solutions; dehydrating solutions (alcohols and xylenes); mounting fluid optional
Examination	Oil immersion examination of at least 300 fields; additional fields may be required if suspect organisms have been seen but are not clearly identified.
Results and Laboratory Reports	The identification of microsporidial spores may be possible; however, their small size will make recognition very difficult. Final laboratory results would depend heavily on the appearance of the QC slides and comparison with patient specimens. Although extruded polar tubes have been seen rarely in clinical samples such as sputum, cerebrospinal fluid, urine, intestinal biopsy specimens, conjunctival swab specimens, and duodenal fluid, they are almost never seen in fecal specimens.
Procedure Notes and Limitations	Because of the difficulty in getting dye to penetrate the spore wall, this staining approach can be very helpful. Procedure limitations are related to specimen handling (proper centrifugation speeds and time, use of no more than two layers of wet gauze for filtration, and complete understanding of the difficulties in recognizing microsporidial spores owing to their small size [1 to 2.0 μm]).
Important Questions for Commercial Suppliers	Make sure to ask about specific fixatives and whether the fecal material can be stained with the modified trichrome stains and modified acid-fast stains. Also, ask if the fixatives prevent the use of any of the newer immunoassay methods now available for several of the intestinal amebae, flagellates, coccidia, and microsporidia (under development).

ment). This is the same method we use for centrifugation of all stool specimens, regardless of the suspected organism.

5. Avoid the use of wet-gauze filtration (an old, standardized method of filtering stool prior to centrifugation) with too many layers of gauze that may trap organisms and not allow them to flow into the fluid to be concentrated. It is recommended that no more than two layers of gauze be used. Another option is to use the commercially available concentration systems in which metal or plastic screens are used for filtration.

Modified Trichrome Stain for the Microsporidia (Kokoskin—Hot Method) (38)

Changes in temperature from room temperature to 50°C and in the staining time from 90 to 10 min have been recommended as improvements for the modified trichrome staining methods. The procedure is as follows.

1. Using a 10-μl aliquot of concentrated, preserved stool (5 or 10% formalin, SAF, or Universal Fixative), prepare the smear by spreading the material over an area 45 by 25 mm.
2. Allow the smear to air dry.
3. Place the smear in absolute methanol for 5 min.
4. Allow the smear to air dry.
5. Place in trichrome stain for 10 min at 50°C.
6. Rinse in acid-alcohol for no more than 10 s.
7. Dip the slide several times in 95% alcohol. Use this step as a rinse (no more than 10 s).
8. Place in 95% alcohol for 5 min.
9. Place in 100% alcohol for 10 min.
10. Place in xylene substitute for 10 min.
11. Mount with a coverslip (no. 1 thickness), using mounting medium.
12. Examine the smear under oil immersion (×1,000) and read at least 300 fields; the examination time will probably be at least 10 min per slide.

Acid-Fast Trichrome Stain for *Cryptosporidium* and the Microsporidia

The detection of *Cryptosporidium* spp. and the microsporidia from stool specimens has depended on two separate stains. However, a method is now available that stains both organisms, an important improvement since dual infections have been demonstrated in AIDS patients (44).

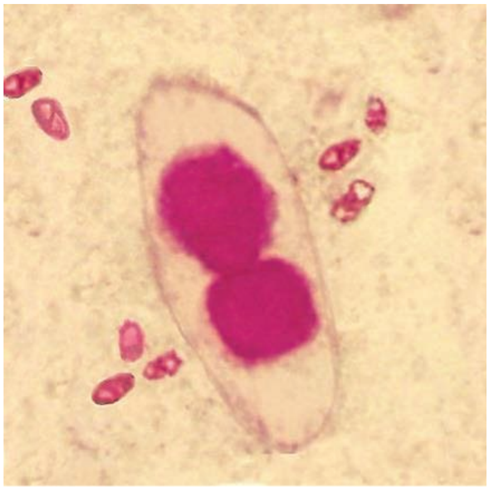


Figure 3.30 *Cystoisospora* (*Isospora*) *belli* oocyst and microsporidian spores in an acid-fast trichrome stain. Note the size differential. doi:10.1128/9781555819002.ch3.f30

This acid-fast trichrome stain yields results comparable to those obtained by the Kinyoun and modified trichrome methods and considerably reduces the time necessary for microscopic examination (30, 45, 46) (Fig. 3.30). Also, it appears that modified trichrome stains and staining with fluorochromes are equally useful in the diagnosis of microsporidiosis; however, a combination of the two methods may be more sensitive in cases where the number of spores is very small (41).

The specimen can be fresh stool or stool that has been preserved in 5 or 10% formalin, SAF, or some of the newer single-vial-system fixatives. Actually, any specimen other than tissue thought to contain microsporidia could be stained by this method.

Trichrome Stain (Modified for Microsporidia) (44)

Chromotrope 2R	6.0 g*
Aniline blue	0.5 g
Phosphotungstic acid	0.7 g
Acetic acid (glacial)	3.0 ml
Distilled water	100.0 ml

*(10 times the normal trichrome stain formula)

1. Prepare the stain by adding 3.0 ml of acetic acid to the dry ingredients. Allow the mixture to stand (ripen) for 30 min at room temperature.
2. Add 100 ml of distilled water, and adjust the pH to 2.5 with 2.0 N HCl. Properly prepared stain is dark purple. The staining solution should be protected from light.
3. Store in a glass or plastic bottle at room temperature. The shelf life is at least 24 months.

Carbol Fuchsin Solution

Phenol solution

Phenol	25.0 g
Distilled water	500.0 ml

Saturated alcoholic fuchsin solution

Basic fuchsin	2.0 g
96% ethanol	25.0 ml

Add the mixture of phenol and water to 25.0 ml of the saturated alcoholic fuchsin solution.

Acid-Alcohol

90% ethyl alcohol	995.5 ml
Acetic acid (glacial)	4.5 ml

Prepare by combining the two solutions.

Quality Control for the Acid-Fast Trichrome Staining Method

1. Unfortunately, the only way to perform acceptable QC procedures for this method is to use actual microsporidian spores as the control organisms. Obtaining these positive controls may be somewhat difficult. It is particularly important to use the actual organisms because the spores are difficult to stain and are very small (1 to 2.5 µm).
2. A QC slide should be included with each run of stained slides, particularly if the staining setup is used infrequently.
3. All staining dishes should be covered to prevent evaporation of reagents (screw-cap Coplin jars or glass lids).
4. Depending on the volume of slides stained, staining solutions must be changed on an as-needed basis.
5. When the smear is thoroughly fixed and the stain is performed correctly, the spores are ovoid and refractile, with the spore wall being bright pinkish red. Occasionally, the polar tube can be seen either as a stripe or as a diagonal line across the spore. The majority of the bacteria and other debris tend to stain blue; however, some bacteria and debris stain red.
6. The specimen is also checked for adherence to the slide (macroscopically).
7. The microscope should be calibrated (within the last 12 months if it has received heavy use), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope). Although recalibration every 12 months may not be necessary, this varies from laboratory to laboratory, depending on equipment care and use.

8. Known positive microscope slides and photographs (reference books) should be available at the workstation.
9. Record all QC results; the laboratory should also have an action plan for "out-of-control" results.

Procedure for the Acid-Fast Trichrome Staining Method

1. Using a 10-µl aliquot of concentrated (10 min at 500 × *g*), preserved liquid stool (5 or 10% formalin, SAF, one of the single-vial preservatives, zinc based or Universal Fixative), prepare the smear by spreading the material over an area 45 by 25 mm (47, 48).
2. Allow the smear to air dry.
3. Place the smear in absolute methanol for 5 or 10 min.
4. Allow the smear to air dry.
5. Place in carbol fuchsin solution for 10 min (no heat required).
6. Rinse briefly with tap water.
7. Decolorize with 0.5% acid-alcohol.
8. Briefly rinse with tap water.
9. Place in trichrome stain for 30 min at 37°C.
10. Rinse in acid-alcohol for no more than 10 s.
11. Dip the slide several times in 95% alcohol. Use this step as a rinse (no more than 10 s).
12. Place in 95% alcohol for 30 s.
13. Allow the slide to air dry.
14. Examine the smear under oil immersion (×1,000) and read at least 300 fields; the examination time will probably be at least 10 min per slide.

Results and Patient Reports from the Acid-Fast Trichrome Staining Method

The microsporidial spore wall should stain pink, with the interior of the spore being clear or perhaps showing a horizontal or diagonal stripe that represents the polar tube; a vacuole may also be visible in some spores. The *Cryptosporidium* oocysts stain bright pink or violet. The results of this staining procedure should be reported only if the positive control smears are acceptable. The production of immunoassay reagents should provide a more specific and sensitive approach to the identification of the microsporidia in fecal specimens.

1. Report the organism.
   Examples: Microsporidial spores present. The following information can also be added to the report to assist the physician in treating and following the patient:
   The organisms are probably *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis* (if from fecal specimens or urine).
   The organisms are probably *Encephalitozoon intestinalis* (identification to species highly likely) (generally the organism involved in disseminated cases from the gastrointestinal tract to kidneys; organisms are recovered in urine).

Procedure Notes for the Acid-Fast Trichrome Staining Method

1. It is mandatory that positive control smears be stained and examined each time patient specimens are stained and examined.
2. Because of the difficulty in achieving stain penetration through the spore wall, prepare thin smears and do not reduce the staining time in trichrome. Also, make sure the slides are not left too long in the decolorizing agent (acid-alcohol). If the control organisms are too light, leave them in the trichrome longer and shorten the time to two dips in the acid-alcohol solution. Also, remember that the 95% alcohol rinse after the acid-alcohol step should be performed quickly to prevent additional destaining from the acid-alcohol reagent.
3. When you purchase the chromotrope 2R, obtain the highest dye content available. Two sources are Harleco, Gibbstown, NJ, and Sigma Chemical Co., St. Louis, MO. (the dye content is among the highest [85%]). Fast green and aniline blue can be obtained from Allied Chemical and Dye, New York, NY. See also appendix 5.
4. In the final stages of dehydration, the 95% ethanol should be kept as free from water as possible. Coplin jars must have tight-fitting caps to prevent both evaporation of reagents and absorption of moisture.

Procedure Limitations for the Acid-Fast Trichrome Staining Method

1. Although this staining method stains the microsporidia, the range of stain intensity and the small size of the spores may still cause some difficulty in identifying these organisms. Since this procedure results in many other organisms or objects staining in stool specimens, differentiation of the microsporidia from surrounding material is still very difficult. There also tends to be some slight size variation among the spores.
2. If the patient has severe watery diarrhea, there is less artifact material in the stool to confuse with the microsporidial spores; however, if the stool is semiformed or formed, the amount of artifact material is much greater and so the spores are much harder to detect and identify. Also, the number of spores may vary according to the stool consistency

(generally, the more liquid the stool, the more spores are present).

3. The workers who developed some of these procedures think that concentration procedures result in an actual loss of microsporidial spores; therefore there is a recommendation to use unconcentrated, formalinized stool. **However, there are no data indicating which centrifugation speeds were used in the study.**
4. In the UCLA Clinical Microbiology Laboratory, we have generated data (unpublished) to indicate that **centrifugation at 500 × *g* for 10 min dramatically increases the number of microsporidial spores available for staining (from the concentrate sediment).** We use the same protocol for centrifugation of all stool specimens, regardless of the suspected organism. The acid-fast trichrome procedure presented here also recommended the use of centrifuged fecal specimens.
5. Avoid the use of wet-gauze filtration (an old, standardized method of filtering stool prior to centrifugation) with too many layers of gauze that may trap organisms and not allow them to flow into the fluid to be concentrated. It is recommended that no more than two layers of gauze be used. Another option is to use the commercially available concentration systems that use metal or plastic screens for filtration.

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The correct answers for Fig. 3.17 are as follows: top row left and right, *Giardia lamblia* (*G. duodenalis, G. intestinalis*) trophozoite, *Chilomastix mesnili* cyst; next row left and right, *Endolimax nana* or *Iodamoeba bütschlii* trophozoite (both look essentially the same in the trophozoite form), *Entamoeba coli* cyst; next row left and right, *Dientamoeba fragilis* trophozoite, *Blastocystis* spp.; next row left and right, *Entamoeba histolytica* trophozoite, *Blastocystis* spp.; bottom row left and right, *Endolimax nana* cyst, *Entamoeba histolytica/E. dispar* trophozoite.

4

Additional Techniques for Stool Examination

Culture of larval-stage nematodes

- Harada-Mori filter paper strip culture
- Filter paper/slant culture technique (petri dish)
- Charcoal culture
- Baermann technique
  - Modification of the Baermann method
- Agar plate culture for *Strongyloides stercoralis*

Egg studies

- Estimation of worm burdens and Kato-Katz thick film
  - Direct-smear method of Beaver
  - Dilution egg count
  - Modified Stoll dilution method
  - Kato-Katz thick smear
- Hatching of schistosome eggs

Search for tapeworm scolex

India ink injection procedure for tapeworm proglottids

Qualitative test for fecal fat

Quantitation of reducing substances (Clinitest)

Among the diagnostic techniques used with stool specimens, the routine ova and parasite (O&P) examination is the best known. This technique has three components: the direct wet mount, the examination of material from a stool concentrate, and the permanent stained smear. This is an excellent procedure and is recommended for most intestinal parasites. However, several other diagnostic techniques are available for the recovery and identification of parasitic organisms. Most laboratories do not routinely offer all of these techniques, but many are relatively simple and inexpensive to perform (1–4). The clinician should be aware of the possibilities and the clinical relevance of information obtained from using such techniques. Occasionally, it is necessary to examine stool specimens for the presence of scolices and proglottids of cestodes and adult nematodes and trematodes to confirm the diagnosis and/or for species identification. A method for the recovery of these stages is also described in this chapter. Although not routinely performed, tests for fecal fat and reducing substances are also included.

Culture of Larval-Stage Nematodes

Nematode infections giving rise to larval stages that hatch in soil or in tissues may be diagnosed by using certain fecal culture methods to concentrate the larvae. *Strongyloides stercoralis* larvae are generally the most common larvae found in stool specimens. However, depending on the fecal transit time through the intestine and the patient's condition, rhabditiform and, rarely, filariform larvae may be present. Also, if there is a delay in examination of the stool, embryonated ova as well as larvae of hookworm may be present. Culture of feces for larvae is useful to (i) reveal their presence when they are too scanty to be detected by concentration methods; (ii) distinguish whether the infection is due to *S. stercoralis* or hookworm on the basis of rhabditiform larval morphology by allowing hookworm egg hatching to occur, releasing first-stage larvae; and (iii) allow the development of larvae into the filariform stage for further differentiation.

The use of certain fecal culture methods (sometimes referred to as coproculture) is especially helpful for detection of light infections with hookworm, *S. stercoralis*, and *Trichostrongylus* spp. and for specific identification of parasites. The rearing of infective-stage nematode larvae also helps in the specific diagnosis of hookworm and trichostrongyle infections because the eggs of many of these species are identical and specific identifications are based on larval morphology.

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Additionally, such techniques are useful for obtaining a large number of infective-stage larvae for research purposes. Four culture techniques and one "enhanced-recovery" method are described in this chapter.

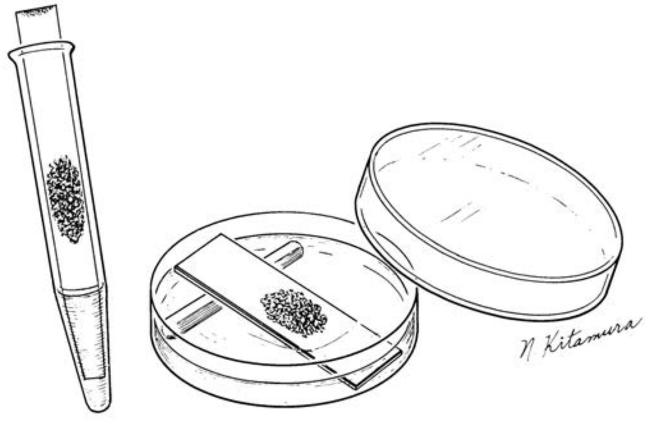
Harada-Mori Filter Paper Strip Culture

To detect light infections with hookworm, *S. stercoralis*, and *Trichostrongylus* spp., as well as to facilitate specific identification, the Harada-Mori filter paper strip culture technique is very useful (Fig. 4.1). This filter paper test tube culture technique was initially introduced by Harada and Mori in 1955 (5) and was later modified by others (6, 7).

In a study looking at the prevalence of *S. stercoralis* in three areas of Brazil, the diagnostic efficacy of the agar plate culture method (discussed later in this chapter) was as high as 94.9% compared to only 28.5 and 26.5% by the Harada-Mori filter paper culture and fecal concentration methods, respectively, when fecal specimens were processed using all three methods (8). Among the 49 positive samples, about 60% were confirmed as positive by using the agar plate method alone. These results indicate that the agar plate approach is probably a much more sensitive diagnostic method than the other two and is recommended for the diagnosis of strongyloidiasis.

The technique requires filter paper to which fresh fecal material is added and a test tube into which the filter paper is inserted. Moisture is provided by adding water to the tube, which continuously soaks the filter paper by capillary action. Incubation under suitable conditions favors hatching of ova and/or development of larvae. Fecal specimens to be cultured should not be refrigerated, since some parasites (especially *Necator americanus*) are susceptible to cold and may fail to develop after refrigeration. *Also, caution must be exercised in handling the filter paper strip itself, since infective* Strongyloides *larvae may migrate upward as well as downward on the paper strip. Always observe standard precautions and wear gloves when performing these procedures.*

Figure 4.1 Culture methods for the recovery of larval-stage nematodes: Harada-Mori tube method and petri dish culture method. Viable larvae are present if the specimen contains *S. stercoralis* or other nematodes. Wear gloves when handling the culture devices. (Illustration by Nobuko Kitamura.)
doi:10.1128/9781555819002.ch4.f1



Quality Control for Harada-Mori Filter Paper Strip Culture

1. Follow routine procedures for optimal collection and handling of fresh fecal specimens for parasitologic examination.
2. Examine known positive and negative samples of stools (from laboratory animals), if available, to gain some experience in using the procedure.
3. Review larval diagrams and descriptions for confirmation of larval identification.
4. The microscope should be calibrated, and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. If the microscope undergoes hard use or is moved around within the laboratory, it is strongly recommended that recalibration be performed every 12 months. However, if the microscope remains in the same location and receives normal use, such frequent recalibration may not be required. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
5. Record all quality control (QC) results.

Procedure for Harada-Mori Filter Paper Strip Culture

1. Smear 0.5 to 1 g of fresh feces in the center of a narrow strip of filter paper (3/8 by 5 in. [1 in. = 2.54 cm], slightly tapered at one end).
2. Add 3 to 4 ml of distilled water to a 15-ml conical centrifuge tube; identify the specimen on the tube.
3. Insert the filter paper strip into the tube so that the tapered end is near the bottom of the tube. The water level should be approximately 1/2 in. below the fecal spot. It is not necessary to cap the tube. However, a cork stopper or a cotton plug may be used.
4. Maintain the tube upright in a rack at 25 to 28°C. Add distilled water to maintain the original level (usually evaporation takes place over the first 2 days, and then the culture becomes stabilized).
5. Keep the tube for 10 days, and check it daily by withdrawing a small amount of fluid from the bottom of the tube. Prepare a smear on a glass slide, cover the slide with a coverslip, and examine the smear with the 10× objective.

6. Examine the larvae for motility and typical morphological features to reveal whether hookworm, *Strongyloides*, or *Trichostrongylus* larvae are present.

Results and Patient Reports from Harada-Mori Filter Paper Strip Culture

Larval nematodes of hookworm, *S. stercoralis*, or *Trichostrongylus* spp. may be recovered. If *Strongyloides* organisms are present, free-living stages and larvae may be found after several days in culture.

1. Report "No larvae detected" if no larvae could be detected at the end of the incubation.
2. Report larvae detected by fecal culture.
   Example: *Strongyloides stercoralis* larvae detected by fecal culture

Procedure Notes for Harada-Mori Filter Paper Strip Culture

1. If the larvae are too active to observe under the microscope and morphologic details are difficult to see, the larvae can be heat killed within the tube or after removal to the slide; iodine can also be used to kill larvae.
2. Infective larvae may be found any time after the fourth day or even on the first day in a heavy infection. Since infective larvae may migrate upward as well as downward on the filter paper strip, *caution must be exercised in handling the fluid and the paper strip itself to prevent infection. Handle the filter paper with forceps. Wear gloves when handling the cultures.*
3. It is important to maintain the original water level to keep optimum humidity.
4. Fresh stool is required for this procedure; preserved fecal specimens or specimens obtained after a barium meal are not suitable.

Procedure Limitations for Harada-Mori Filter Paper Strip Culture

1. The Harada-Mori technique allows both parasitic and free-living forms of nematodes to develop. If specimens have been contaminated with soil or water containing these forms, it may be necessary to distinguish parasitic from free-living forms. This distinction is possible since parasitic forms are more resistant to slight acidity than are free-living forms. Proceed as follows (9, 10).

   Add 0.3 ml of concentrated hydrochloric acid per 10 ml of water containing the larvae (adjust the volume accordingly to achieve a 1:30 dilution of acid). Free-living nematodes are killed, while parasitic species live for about 24 h.
2. Specimens that have been refrigerated or preserved are not suitable for culture. Larvae of certain species are susceptible to cold environments.

Filter Paper/Slant Culture Technique (Petri Dish)

An alternative technique for culturing *Strongyloides* larvae is a filter paper/slant culture on a microscope slide placed in a glass or plastic petri dish (Fig. 4.1), which was originally described by Little (11). As with previous techniques, sufficient moisture is provided by continuous soaking of the filter paper in water. Fresh stool material is placed on the filter paper, which is cut to fit the dimensions of a standard (1 by 3 in.) microscope slide. The filter paper is then placed on a slanted glass slide in a glass or plastic petri dish containing water. This technique allows direct examination of the culture system with a dissecting microscope to look for nematode larvae and free-living stages of *S. stercoralis* in the fecal mass or the surrounding water without having to sample the preparation. *Always wear gloves when performing these procedures.*

Quality Control for the Filter Paper/Slant Culture Technique (Petri Dish)

1. Follow routine procedures for optimal collection and handling of fresh fecal specimens for parasitologic examination.
2. Examine known positive and negative samples of stools (from laboratory animals), if available, to make sure that the procedure is precise.
3. Review larval diagrams and descriptions for confirmation of larval identification.
4. The microscope should be calibrated, and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
5. Record all QC results.

Procedure for the Filter Paper/Slant Culture Technique (Petri Dish)

1. Cut a filter paper strip (1 by 3 in.), and smear a film of 1 to 2 g of fresh fecal material in the center of the strip.
2. Place the strip on a glass slide (1 by 3 in.). Place the slide inclined at one end of the petri dish by resting the slide on a piece of glass rod or glass tubing; identify the specimen on the dish (Fig. 4.1).
3. Add water to the petri dish so that at least the bottom one-fourth of the slide is immersed in water.

The stool will be kept moist by capillary action. Cover the dish, and maintain it at 25 to 28°C. As needed, add water to maintain the original level.

4. Keep the dish for 10 days. Examine daily, either with the dissecting microscope or by withdrawing a small amount of fluid and placing it on a microscope slide. Cover with a coverslip, and examine microscopically with the 10× and 40× objectives.
5. Examine any larvae recovered for typical morphologic features.

Results and Patient Reports from the Filter Paper/Slant Culture Technique (Petri Dish)

Larval nematodes of hookworm, *S. stercoralis*, or *Trichostrongylus* spp. may be recovered. If *Strongyloides* organisms are present, free-living stages and larvae may be found after several days in culture.

1. Report "No larvae detected" if no larvae could be detected at the end of incubation.
2. Report larvae detected by fecal culture.
   Example: *Strongyloides stercoralis* larvae detected by fecal culture

Procedure Notes for the Filter Paper/Slant Culture Technique (Petri Dish)

1. It is often difficult to observe details in rapidly moving larvae; a drop of iodine or formalin or slight heating can be used to kill the larvae.
2. Infective larvae may be found any time after the fourth day and occasionally after the first day in heavy infections. Since infective larvae may migrate anywhere on the filter paper strip, *caution must be exercised in handling the fluid and the paper strip itself to prevent infection. Wear gloves when handling the cultures*.
3. There may be infective larvae in the moisture that accumulates under the petri dish lid, so be careful not to allow the water to touch the skin when raising the lid.
4. It is important to maintain the original water level to keep optimum humidity.
5. Preserved fecal specimens or specimens obtained after a barium meal are not suitable; fresh stool specimens must be used.

Procedure Limitations for the Filter Paper/Slant Culture Technique (Petri Dish)

1. The filter paper/slant culture technique allows both parasitic and free-living forms of nematodes to develop. If specimens have been contaminated with soil or water containing these forms, it may be necessary to distinguish parasitic from free-living forms. This distinction is possible since parasitic forms are more resistant to slight acidity than are free-living forms. Proceed as follows (9, 10).

   Add 0.3 ml of concentrated hydrochloric acid per 10 ml of water containing the larvae (adjust the volume accordingly to achieve a 1:30 dilution of acid). Free-living nematodes are killed, while parasitic species live for about 24 h.
2. Specimens that have been refrigerated or preserved are not suitable for culture. Larvae of certain species are susceptible to cold environments.

Charcoal Culture

Another way to culture hookworm, *Strongyloides*, and trichostrongyle larvae is by using a granulated charcoal culture. The conditions of this culture provide an environment for larval development that mimics conditions in nature. It provides an efficient way to harvest large numbers of infective-stage larvae for use in experimental infections.

Quality Control for Charcoal Culture

1. Follow routine procedures for optimal collection and handling of fresh fecal specimens for parasitologic examination.
2. Examine known positive and negative samples of stools (from laboratory animals), if available, to make sure that the procedure is precise.
3. Review larval diagrams and descriptions for confirmation of larval identification.
4. The microscope should be calibrated, and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
5. Record all QC results.

Procedure for Charcoal Culture

1. Mix 20 to 40 g of fresh fecal material in tap water until a thick suspension is obtained.
2. Add this suspension to a storage dish (4 by 3 in.) that is slightly more than half filled with no. 10 granulated hardwood charcoal. Mix thoroughly with a wooden tongue depressor until the fecal suspension is evenly distributed throughout the moistened charcoal. Water can be added to ensure that there is adequate moisture, but do not add so much water than it forms a layer on the bottom of the dish. The surface of the charcoal should glisten.
3. Cover the dish, and place it in the dark (in a drawer or cabinet).

4. Check the dish the next day to make sure that there is still sufficient moisture (i.e., that the charcoal still glistens); if water is needed, sprinkle it on the surface without further mixing.
5. Check the dish each day for moisture content. *Caution must be used because moisture will accumulate on the underface of the lid, and it may contain infective-stage larvae.*
6. Approximately 5 or 6 days after the culture has been prepared, hookworm and *Strongyloides* larvae will have reached the infective stage. This can occur earlier if the patient has a heavy infection with numerous larvae in the stool.
7. To harvest the larvae, prepare a round gauze pad of 10 to 12 layer thickness stapled at the edges and cut to fit the dish. Moisten the pad (not dripping wet), and apply it carefully with forceps so that it snugly covers the surface of the charcoal. *Do not allow your hands to touch the charcoal—the larvae will be infective!*
8. Expose the dish, with lid off, to a light source such as a gooseneck lamp. The lamp should be 6 to 8 in. from the surface of the charcoal. Make sure the lamp is not too close; the larvae can be killed by the heat.
9. After approximately 1 h, the pad can be carefully removed with forceps and inverted onto the surface of water in a pilsner glass filled with water. The gauze pad will remain at the top, and the larvae will now make their way through the pad, enter the water, and fall to the bottom of the glass, where they can be harvested with a pipette after another 30 to 60 min. With care, there will be no charcoal at the bottom of the glass, and the larvae will form a clean sediment.

Results and Patient Reports from Charcoal Culture

Larval nematodes of hookworm, *S. stercoralis*, or *Trichostrongylus* spp. may be recovered. If *Strongyloides* organisms are present, free-living stages and larvae may be found after several days in culture.

1. Report "No larvae detected" if no larvae could be detected at the end of incubation.
2. Report larvae detected by fecal culture.
   Example: *Strongyloides stercoralis* larvae detected by fecal culture

Procedure Notes for Charcoal Culture

1. It is often difficult to observe details in rapidly moving larvae; a drop of iodine or formalin or slight heating can be used to kill the larvae.
2. Infective larvae may be found any time after the fourth day and occasionally after the first day in heavy infections. Since infective larvae may be present, *use caution when handling the fluid, gauze pad, and charcoal to prevent infection. Wear gloves when handling the cultures.*
3. It is important to maintain the moisture on the charcoal to keep optimum humidity (the charcoal should glisten).
4. Preserved fecal specimens or specimens obtained after a barium meal are not suitable; fresh stool specimens must be obtained.

Procedure Limitations for Charcoal Culture

1. This technique allows both parasitic and free-living forms of nematodes to develop. If specimens have been contaminated with soil or water containing these forms, it may be necessary to distinguish parasitic from free-living forms. This distinction is possible since parasitic forms are more resistant to slight acidity than are free-living forms. Proceed as follows (9, 10).

   Add 0.3 ml of concentrated hydrochloric acid per 10 ml of water containing the larvae (adjust the volume accordingly to achieve a 1:30 dilution of acid). Free-living nematodes are killed, while parasitic species live for about 24 h.
2. Specimens that have been refrigerated or preserved are not suitable for culture. Larvae of certain species are susceptible to cold environments.

Baermann Technique

Another method of examining a stool specimen suspected of containing small numbers of *Strongyloides* larvae is the use of a modified Baermann apparatus (Fig. 4.2). The Baermann technique, in which a funnel apparatus is used, relies on the principle that active larvae will migrate from a fresh fecal specimen that has been placed on a wire mesh with several layers of gauze which are in contact with tap water (1, 12). Larvae migrate through the gauze into the water and settle to the bottom of the funnel, where they can be collected and examined. The main difference between this method and the Harada-Mori and petri dish methods is the greater amount of fresh stool used, possibly providing a better chance of larval recovery in a light infection. Besides being used for patient fecal specimens, this technique can be used to examine soil specimens for the presence of larvae.

Quality Control for the Baermann Technique

1. Follow routine procedures for optimal collection and handling of fresh specimens for parasitologic examination.
2. Examine known positive and negative samples of stools (from laboratory animals), if available, to make sure that the procedure is precise.

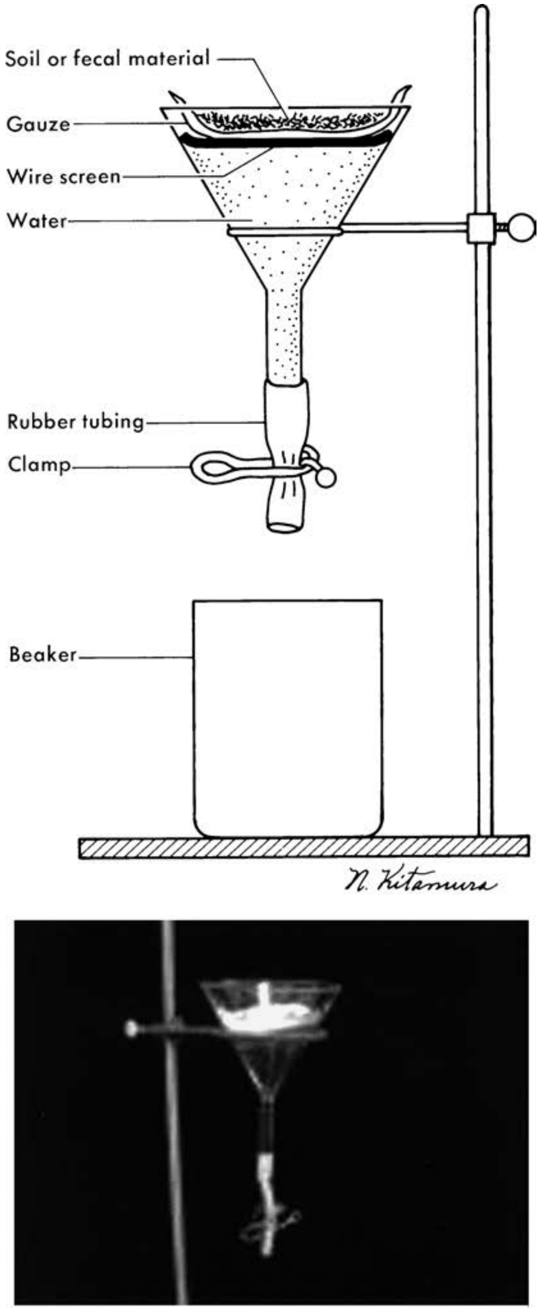


Figure 4.2 (Upper) Baermann apparatus. (Illustration by Nobuko Kitamura.) (Lower) Baermann apparatus set up in the laboratory. doi:10.1128/9781555819002.ch4.f2

3. Review larval diagrams for confirmation of larval identification.
4. The microscope should be calibrated, and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
5. Record all QC results.

Procedure for the Baermann Technique

1. If possible, use a fresh fecal specimen that has been obtained after administration of a mild saline cathartic, not a stool softener. Soft stool is recommended; however, any fresh fecal specimen is acceptable.
2. Set up a clamp supporting a 6-in. glass funnel. Attach rubber tubing and a pinch clamp to the bottom of the funnel. Place a collection beaker underneath (Fig. 4.2).
3. Place a wire gauze or nylon filter over the top of the funnel, followed by a pad consisting of two layers of gauze.
4. Close the pinch clamp at the bottom of the tubing, and fill the funnel with tap water until it just soaks the gauze padding.
5. Spread a large amount of fecal material on the gauze padding so that it is covered with water. If the fecal material is very firm, first emulsify it in water.
6. Allow the apparatus to stand for 2 h or longer; then draw off 10 ml of fluid into the beaker by releasing the pinch clamp, centrifuge the fluid for 2 min at 500 × *g*, and examine the sediment under the microscope (magnification, ×100 and ×400) for the presence of motile larvae. *Make sure that the end of the tubing is well inside the beaker before slowly releasing the pinch clamp. Infective larvae may be present; wear gloves when performing this procedure.*

Results and Patient Reports from the Baermann Technique

Larval nematodes (hookworm, *S. stercoralis*, or *Trichostrongylus* spp.) may be recovered. Both infective and noninfective *Strongyloides* larvae may be recovered, particularly in a heavy infection.

1. Report "No larvae detected" if no larvae could be detected at the end of incubation.
2. Report larvae detected by fecal culture.
   Example: *Strongyloides stercoralis* larvae detected
   by fecal culture

Procedure Notes for the Baermann Technique

1. It may be difficult to observe morphologic details in rapidly moving larvae; a drop of iodine or formalin or slight heating can be used to kill the larvae.
2. Infective larvae may be found any time after the fourth day and occasionally after the first day in heavy infections. *Caution must be exercised in han-*

dling the fluid, gauze pad, and beaker to prevent infection. Wear gloves when using this technique.

3. Remember to make sure that the pinch clamp is tight until you want to release some of the water.
4. Preserved fecal specimens or specimens obtained after a barium meal are not suitable for processing by this method; fresh stool specimens must be obtained.

Procedure Limitations for the Baermann Technique

1. The Baermann technique allows both parasitic and free-living forms of nematodes to develop. If specimens have been contaminated with soil or water containing these forms, it may be necessary to distinguish parasitic from free-living forms. This distinction is possible since parasitic forms are more resistant to slight acidity than are free-living forms. Proceed as follows (9, 10).

   Add 0.3 ml of concentrated hydrochloric acid per 10 ml of water containing the larvae (adjust the volume accordingly to achieve a 1:30 dilution of acid). Free-living nematodes are killed, while parasitic species live for about 24 h.
2. Specimens that have been refrigerated or preserved are not suitable for culture. Larvae of certain species are susceptible to cold environments.
3. *Gloves should be worn when this procedure is performed.*
4. Release the pinch clamp slowly to prevent splashing; have the end of the tubing close to the bottom of the beaker for the same reason.

Modification of the Baermann Method

A simple modification of the Baermann method for diagnosis of strongyloidiasis has been developed (13). For this modification, the funnel used in the original version is replaced by a test tube with a rubber stopper, perforated to allow insertion of a plastic pipette tip (Fig. 4.3). The tube containing the fecal suspension is inverted over another tube containing 6 ml of saline solution and incubated at 37°C for at least 2 h. The saline solution from the second tube is centrifuged, and the pellet is observed microscopically as a wet mount. Larvae of *S. stercoralis* can be found in the pellet. Although the method is almost identical to the original Baermann method, the amount of stool used in the modified method is smaller.

Agar Plate Culture for *Strongyloides stercoralis*

Agar plate cultures are also recommended for the recovery of *S. stercoralis* larvae and tend to be more sensitive than some of the other diagnostic methods (14–22). It is important to remember that more than half of *S. stercoralis*-infected individuals tend to have low-level infections (23). The agar plate method continues to be documented as a more sensitive method than the usual direct smear or fecal concentration methods (16–19). Daily search for furrows on agar plates for up to six consecutive days results in increased sensitivity for diagnosis of both *S. stercoralis* and hookworm infections. Also, a careful search for *S. stercoralis* should be performed for all patients with comparable clinical findings before a diagnosis of idiopathic eosinophilic colitis is made, because the consequent steroid treatment may have a fatal outcome by inducing widespread dissemination of the parasite (24). Human T-cell leukemia virus type 1 (HTLV-1) infection is endemic in a number of Latin American countries. HTLV-1-associated myelopathy/tropical spastic paraparesis and adult T-cell leukemia-lymphoma are emerging diseases in the region. *S. stercoralis* hyperinfection syndrome and therapeutic failure in apparently healthy patients with nondisseminated strongyloidiasis may be markers of HTLV-1 infection (25).

Stool is placed onto agar plates, and the plates are sealed to prevent accidental infections and held for 2 days at room temperature. As the larvae crawl over the agar, they carry bacteria with them, thus creating visible tracks over the agar (Fig. 4.4). The plates are examined under

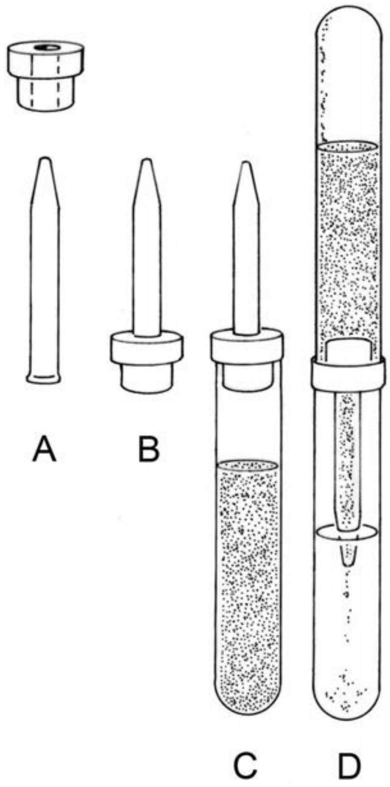


Figure 4.3 Baermann apparatus modification. (Drawing by Sharon Belkin; adapted from reference 13.) doi:10.1128/9781555819002.ch4.f3

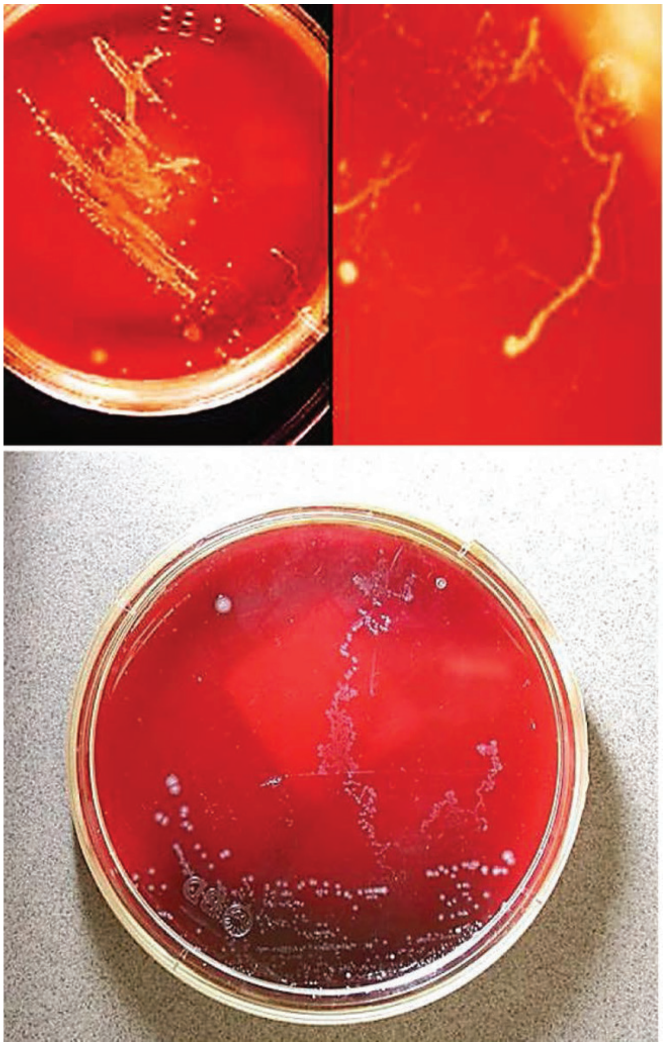


Figure 4.4 Agar plate culture method for *Strongyloides stercoralis*. (Upper) Agar plate showing bacterial growth after being distributed over the plate by the movement of the larval worms. (Lower) More random pattern of bacterial growth from the inoculation of bacteria over the agar from the movement of the larval worms (image courtesy of Audrey N. Schuetz, Weill Cornell Medical College). doi:10.1128/9781555819002.ch4.f4

the microscope for confirmation of the presence of larvae, the surface of the agar is then washed with 10% formalin, and final confirmation of larval identification is made via wet examination of the sediment from the formalin washings (Fig. 4.5).

Occasionally, finding nematode larvae in sputum or bronchoalveolar lavage fluid specimens may be very suggestive of a potential infection with *S. stercoralis*. In Fig. 4.6, larvae can be seen stained with Giemsa stain or a Gram stain. Once larvae are seen in respiratory specimens, fecal specimens can be collected for agar plate cultures to confirm strongyloidiasis.

Agar

1.5% agar
0.5% meat extract
1.0% peptone
0.5% NaCl

Note Positive tracking on agar plates has been seen on a number of different types of agar. However, the most appropriate agar formula is that listed above.

Quality Control for Agar Plate Culture for *Strongyloides stercoralis*

1. Follow routine procedures for optimal collection and handling of fresh fecal specimens for parasitologic examination.
2. Examine agar plates to ensure that there is no cracking and the agar pour is sufficient to prevent drying. Also, make sure that there is no excess water on the surface of the plates.
3. Review larval diagrams and descriptions for confirmation of larval identification.
4. The microscope should be calibrated, and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
5. Record all QC results (condition of agar plates).

Procedure for Agar Plate Culture for *Strongyloides stercoralis*

1. Place approximately 2 g of fresh stool in the center of the agar plate (area approximately 1 in. in diameter).
2. Replace the lid, and seal the plate with cellulose tape.
3. Maintain the agar plate (right side up) at room temperature for 2 days.
4. After 2 days, examine the sealed plates through the plastic lid under the microscope for microscopic colonies that develop as random tracks on the agar and evidence of larvae at the ends of the tracks away from the stool.

   Note It has been documented that daily search for tracks on agar plates for up to six consecutive days results in increased sensitivity for diagnosis of both *S. stercoralis* and hookworm infections (17). When trying to rule out strongyloidiasis in immunocompromised patients or in those who may receive immunosuppressive drugs, it is recommended that two plates be set up, one that can be examined after 2 days and one that can be examined after the full 6 days.

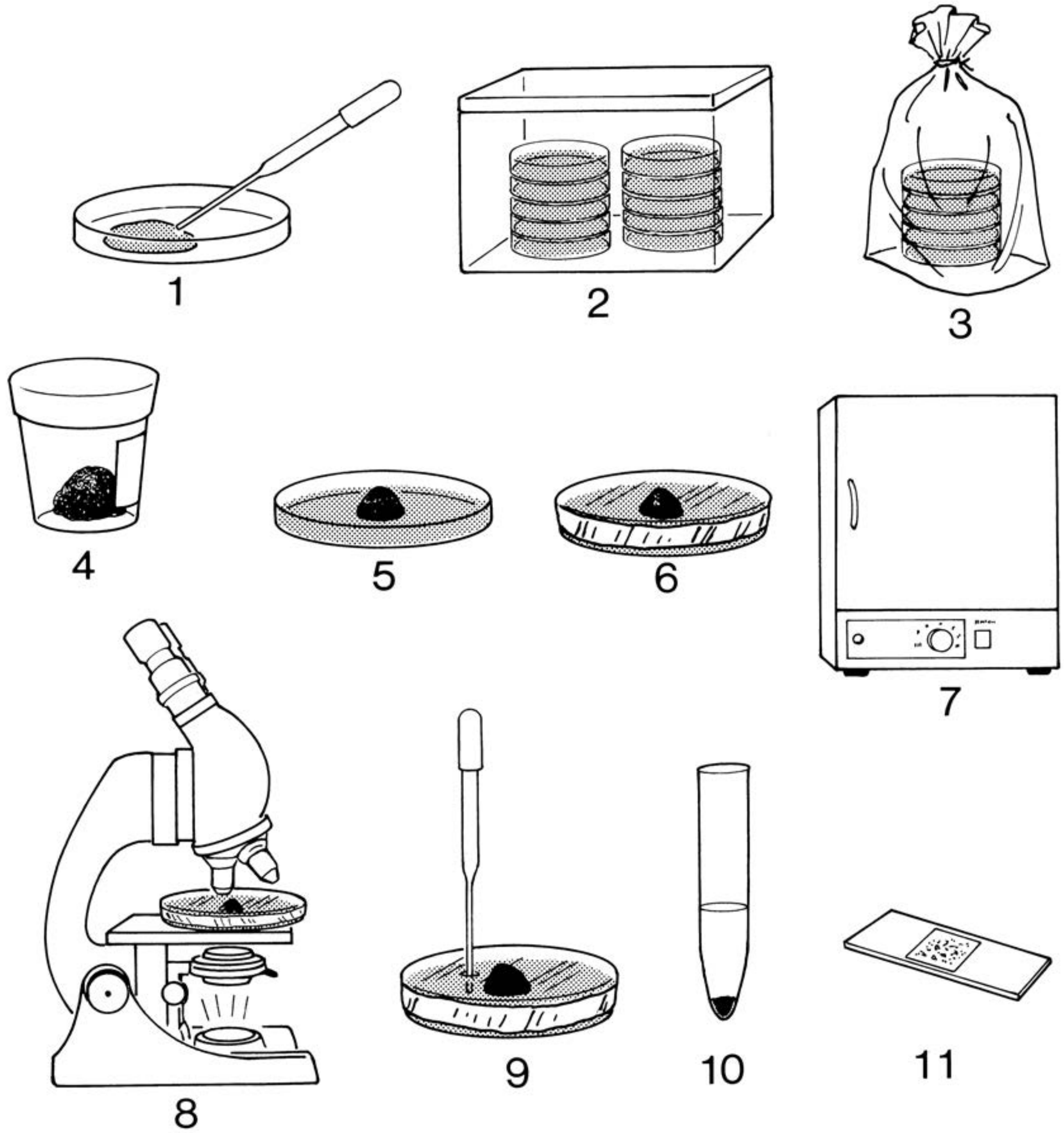


Figure 4.5 Agar culture method for *Strongyloides stercoralis*. (1) Agar plates are prepared. (2) Agar is dried for 4 to 5 days on the bench top. (3) Plates are stored in plastic bags. (4) Fresh stool is submitted to the laboratory. (5) Approximately 2 g of stool is placed onto an agar plate. (6) The plate is sealed with tape. (7) The culture plate is incubated at 26 to 33°C for 2 days. (8) The plate is examined microscopically for the presence of tracks (bacteria carried over agar by migrating larvae). (9) 10% formalin is placed onto agar through a hole made in the plastic with hot forceps. (10) Material from the agar plate is centrifuged. (11) The material is examined as a wet preparation for rhabditiform or filariform larvae (high dry power; magnification, ×400). (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch4.f5

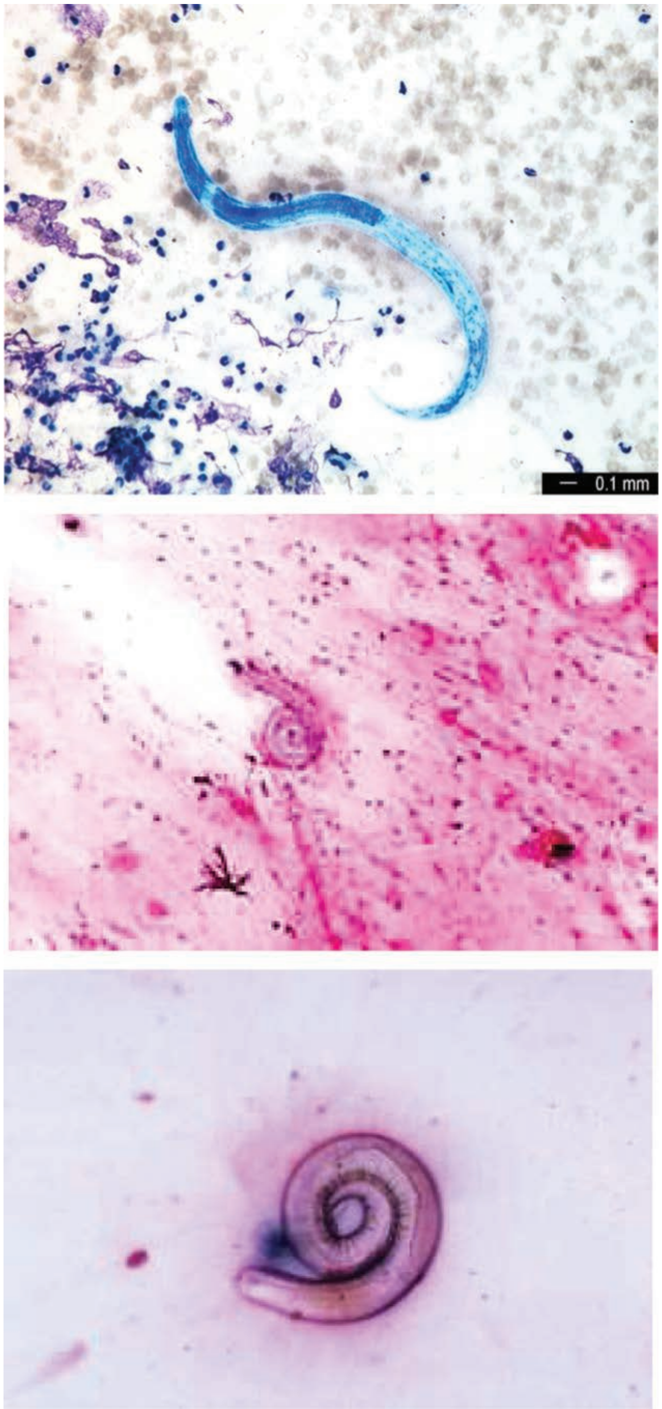


Figure 4.6 *Strongyloides stercoralis* larvae. (Upper) Giemsa-stained larvae in a specimen of bronchoalveolar lavage fluid (courtesy of Marc Long). (Middle) Gram-stained larva in thoracic fluid. (Lower) Gram-stained larva in a respiratory specimen (sputum). doi:10.1128/9781555819002.ch4.f6

5. With the ends of hot forceps, make a hole in the top of the plastic petri dish.
6. Gently add 10 ml of 10% formalin through the hole onto the agar surface, and swirl to cover the surface and rinse the agar plate. Allow to stand for 30 min.
7. Remove the tape and lid of the agar plate. Pour the 10% formalin through a funnel into a centrifuge tube. Do not try and pour the formalin off directly into the centrifuge tube—the tube opening is too small, and formalin will be spilled onto the counter.
8. Centrifuge the formalin rinse fluid 5 min at 500 × *g*.
9. Prepare a wet smear preparation from the sediment and examine with a 10× objective (low power) for presence of larvae. If larvae are found, confirm the identification with a 40× objective (high dry power).

Results and Patient Reports from Agar Plate Culture for *Strongyloides stercoralis*

Larval nematodes of hookworm, *S. stercoralis*, or *Trichostrongylus* spp. may be recovered. If *Strongyloides* organisms are present, free-living stages and larvae may be found after several days on the agar plates.

1. Report "No larvae detected" if no larvae could be detected at the end of incubation and rinse procedure.
2. Report larvae detected by agar plate culture.
   Example: *Strongyloides stercoralis* larvae detected by agar plate culture.

Procedure Notes for Agar Plate Culture for *Strongyloides stercoralis*

1. If the larvae are too difficult to observe under the microscope and morphologic details are difficult to see, the larvae can be formalin killed within the plate and examined in the formalin-concentrated sediment.
2. Infective larvae may be found any time after the first or second day or even on the first day in a heavy infection. Since infective larvae may be present on the agar, *caution must be exercised in handling the plates once the cellulose tape is removed. Wear gloves when handling the cultures*.
3. It is important to maintain the plates upright at room temperature. Do not incubate or refrigerate them at any time; this also applies to the fresh stool specimen.
4. Fresh stool is required for this procedure; preserved fecal specimens or specimens obtained after a barium meal are not suitable.

REVIEW Culture of Larval-Stage Nematodes

Topic	Comments
Principle	Culture of feces for larvae is useful to (i) reveal their presence when they are too scanty to be detected by concentration methods; (ii) distinguish whether the infection is due to *S. stercoralis* or hookworm on the basis of rhabditiform larval morphology by allowing hookworm egg hatching to occur, releasing first-stage larvae; and (iii) allow development of larvae into the filariform stage for further differentiation.
Specimen	Any stool specimen that is fresh and has not been refrigerated can be used.
Examination	Daily checking of the fluid for the presence of larvae; hold the cultures for 10 days before submitting the final report. Agar plate cultures can be held for 6 days. When trying to rule out strongyloidiasis in immunocompromised patients or in those who may receive immunosuppressive drugs, it is recommended that two plates be set up, one that can be examined after 2 days and one that can be examined after the full 6 days.
Results and Laboratory Reports	The failure to recover larvae does not completely rule out the possibility of infection; however, the probability of infection is very low when results are negative.
Procedure Notes and Limitations	There is always the prospect of recovering infective larvae; *gloves must be worn at all times when performing these procedures and carrying out fluid examinations*. Make sure that the culture systems are kept hydrated; a certain amount of water will evaporate and be lost as a result of culture equilibration, particularly during the first couple of days.

Procedure Limitations for Agar Plate Culture for *Strongyloides stercoralis*

1. The agar plate culture technique is successful if any larvae present are viable. If the fresh stool specimen is too old, larvae may not survive and a negative result will be reported.
2. Specimens that have been refrigerated or preserved are not suitable for culture. Larvae of certain species are susceptible to cold environments.

Egg Studies

Estimation of Worm Burdens and Kato-Katz Thick Film

The only human parasites for which it is reasonably possible to correlate egg production with adult worm burdens are *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). The specific instances in which information on approximate worm burdens is useful are when one is determining the intensity of infection, deciding on possible chemotherapy, and evaluating the efficacy of the drugs administered. With current therapy, the need for monitoring therapy through egg counts is no longer as relevant. However, several methods that can be used if necessary are discussed below. **Remember that egg counts are estimates; you will obtain count variations regardless of how carefully you follow the procedure.** If two or more fecal specimens are being compared, it is best to have the same individual perform the technique on both samples and to do multiple counts.

Direct-Smear Method of Beaver

The direct-smear method of Beaver is the easiest to use and is reasonably accurate when performed by an experienced technologist. In the original method, Beaver (26) used a calibrated photoelectric cell to prepare a direct smear of exactly 2 mg. For routine purposes, this is impractical, and the procedure has subsequently been modified (27) such that a direct smear of 2 mg (enough fresh fecal material to form a low cone on the end of a wooden applicator stick) of stool is prepared. Egg counts on the direct smear are reported as eggs per smear, and the appropriate calculations can be made to determine the number of eggs per gram of stool.

Dilution Egg Count

The Stoll count (28) is probably the most widely used dilution egg-counting procedure for the purpose of estimating worm burdens. However, because of cost containment and clinical relevance (therapy is often initiated with no egg count data), most laboratories do not offer this procedure.

Stool displacement flasks for use in this procedure are available commercially. These flasks have a long neck with etched lines at 56 and 60 ml to facilitate proper filling with sodium hydroxide and fecal material. If commercial Stoll flasks are unavailable, any flask that can hold the sodium hydroxide solution, a weighed amount of 4 g of stool, and a few small glass beads can be used for a container.

1. In a calibrated Stoll flask, add 0.1 N sodium hydroxide to the 56-ml mark.
2. Add fresh fecal material to the flask so that the level of fluid rises to the 60-ml mark. This amount of feces is equivalent to 4 g of feces.

3. Add a few glass beads, and shake vigorously to make a uniform suspension. If the specimen is hard, the mixture may be placed in a refrigerator overnight before shaking to aid in mixing.
4. With a calibrated pipette, quickly remove 0.15 ml of suspension and transfer it to a slide.
5. Do not use a coverslip; place the slide on a mechanical stage, and count all of the eggs.
6. Multiply the egg count by 100 to obtain the number of eggs per gram of stool.
7. The estimate (eggs per gram) obtained will vary according to the consistency of the stool. The following correction factors should be used to convert the estimate to a formed-stool basis:

mushy formed	×1.5
mushy	×2
mushy diarrheic	×3

Egg counts on liquid specimens are generally unreliable; the most accurate counts are obtained with use of formed or semiformed specimens.

Modified Stoll Dilution Method

1. Fill a 15-ml centrifuge tube to the 14-ml mark with 0.1 N sodium hydroxide.
2. Add stool to bring the liquid contents up to the 15-ml mark.
3. Mix thoroughly with a wooden applicator stick.
4. If the stool is hard, allow the mixture to stand for several hours.
5. Shake to thoroughly mix, and quickly withdraw exactly 0.15 ml from the middle of the suspension.
6. Transfer the material to a slide, cover (it may be easier to count without a coverslip, since occasionally the eggs flow to the outside of the coverslip), and count the eggs in the entire preparation.
7. Multiply the egg count per preparation by 100 to give an uncorrected count of eggs per milliliter. Corrections may be made as in the Stoll dilution method for original stool consistency.

Kato-Katz Thick Smear

Although this method of counting helminth eggs has been modified many times since it was originally published, the protocol seen below provides an acceptable approach (29). Currently, the Kato-Katz is the recommended method of WHO, with the McMaster method being considered an alternative for the quantification of soil-transmitted helminths eggs in human stool (primarily *Ascaris*, *Trichuris*, hookworm, *Schistosoma mansoni*) (35). Although this thick film method has been adapted to estimate worm burden calculations, it is rarely used in routine clinical laboratories. Also, it is unsuitable for the diagnosis of protozoa or helminth larvae and is difficult to perform on hard and/or liquid stools.

1. Coverslips made of wettable cellophane, medium thickness, cut into 22 × 30 mm rectangles (No. 124 PD, E.I. DuPont de Nemours, Inc., Film Department, Wilmington, DE).
2. Glycerine-malachite green solution: 10 ml pure glycerine and 1 ml 3% aqueous malachite green in 100 ml distilled water. Allow the cellophane coverslips to soak in the glycerine mixture for at least 24 h. Although the malachite green is recommended, it is not essential for the procedure.
3. Some procedures require a metal or synthetic fiber filter mesh; however, this method is more complex. Just remember that if the fecal specimen is formed, hard, and somewhat dry, you will have to add a few drops of water so the final stool/water emulsion becomes mushy. To obtain 10-, 20-, or 50-mg samples, metal templates containing holes calibrated to deliver these fecal amounts can be used.

Procedure

1. Using an applicator stick, transfer approximately 50 mg of fresh stool to a standard 1 × 3-in. microscope slide. (A 4-mm cube of feces weighs about 65 mg.)
2. Cover the stool with the cellophane coverslip.
3. Turn the preparation upside down on a paper towel (flat, absorbent surface), and press it down until the fecal film covers approximately 20 to 25 mm in diameter. If the amount of feces is too much, the excess usually flows from under the cellophane coverslip and is absorbed by the paper towel.
4. Allow the preparation to stand for 1 h at room temperature to allow clearing of the fecal material but not the eggs. Do not overclear; thin-shelled eggs (hookworm) will tend to collapse and may disappear. Examine the slide at the end of the clearing period (usually not to exceed 1 h). It has been recommended that the clearing time be extended to 24 h for *S. mansoni* eggs; however, hookworm eggs will probably have collapsed and disappeared after this amount of time.
5. Examine the entire fecal film using the low power objective (10×).

Correlation with Treatment

The following numbers have been compiled to indicate the correlation between egg counts and the need for treatment. The two helminths listed below are generally the only ones for which the egg count will determine whether the patient is treated for the initial infection. As mentioned above, with the current therapeutic agents available, this information has become less clinically relevant, at least in many parts of the world. However, it has been recognized that egg counts do not always correlate with or accurately predict the worm burden of the host.

1. For *T. trichiura*, about 30,000 eggs/g indicates the presence of several hundred worms, which may cause definite symptoms.
2. For hookworm, about 2,500 to 5,000 eggs/g usually indicates a clinically significant infection.

The effectiveness of therapy for any helminth infection may be evaluated by doing repeated egg counts after treatment. Low egg counts for *T. trichiura* and hookworm are generally reflected by a lack of clinical signs and symptoms in individuals harboring these parasites. However, the presence of even one *Ascaris* worm is potentially dangerous because of the active migrating habits of this parasite, which may result in serious clinical manifestations.

Hatching of Schistosome Eggs

When schistosome eggs (Fig. 4.7) are recovered from either urine or stool, they should be carefully examined to determine viability. The presence of living miracidia within the eggs indicates an active infection that may require therapy. The viability of the miracidia can be determined in two ways: (i) the cilia of the flame cells (primitive excretory cells) may be seen on a wet smear by using high dry power and are usually actively moving, and (ii) the miracidia may be released from the eggs by a hatching procedure (Fig. 4.8) (1, 28, 30, 31). The eggs usually hatch within several hours when placed in 10 volumes of dechlorinated or spring water (hatching may begin soon after contact with the water). The eggs that are recovered in the urine (24-h specimen collected with no preservatives) are easily obtained from the sediment and can be examined under the microscope to determine viability. McMullen and Beaver (31) recommended the use of a sidearm flask, but an Erlenmeyer flask is an acceptable substitute.

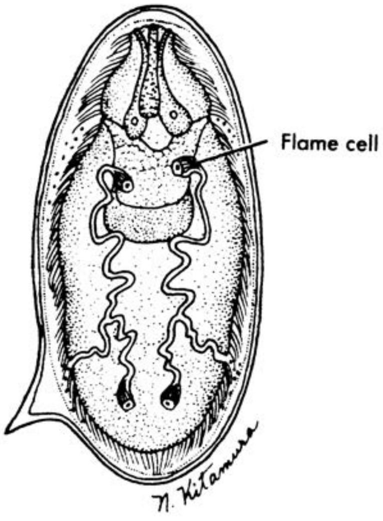


Figure 4.7 *Schistosoma mansoni* egg showing flame cells. (Illustration by Nobuko Kitamura; modified from E. C. Faust et al., *Craig and Faust's Clinical Parasitology*, 8th ed., Lea & Febiger, Philadelphia, PA, 1970.) doi:10.1128/9781555819002.ch4.f7

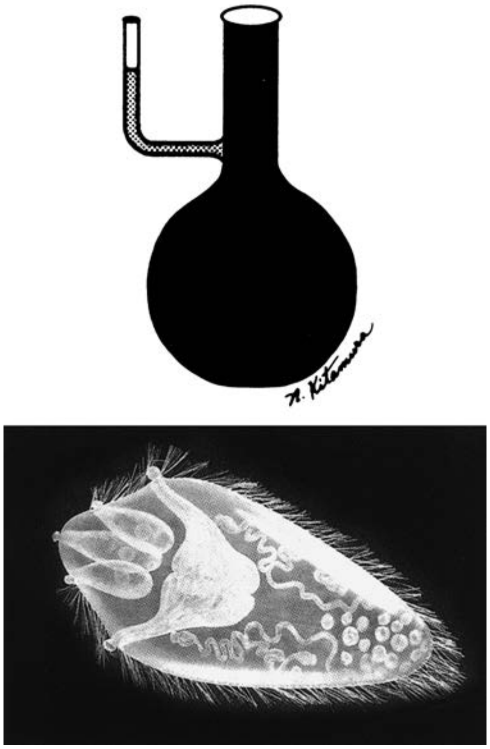


Figure 4.8 (Upper) Sidearm hatching flask used to recover miracidia from viable schistosome eggs (illustration by Nobuko Kitamura). (Lower) Miracidium larva released from egg during hatching procedure (Armed Forces Institute of Pathology photograph). doi:10.1128/9781555819002.ch4.f8

Quality Control for Hatching of Schistosome Eggs

1. Make sure that the water used is chlorine free; chlorine kills the miracidia. You can use bottled water or leave tap water in an open pan overnight before use (eliminates chlorine in tap water).
2. Check the saline solution (used to prepare the stool concentration) for the presence of any free-living organisms (flagellates or ciliates). This is normally not a problem.
3. Since it is neither realistic nor practical for the majority of laboratories to perform parallel positive-control procedures, review drawings and size measurements of schistosome eggs and/or miracidia.

4. The microscope should be calibrated, and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope).
5. Record all QC results.

Procedure for Hatching Schistosome Eggs

1. Thoroughly homogenize a fresh stool specimen (40 to 50 g) in 50 to 100 ml of 0.85% NaCl.
2. Strain through two layers of gauze placed on a funnel. Collect material in a small beaker or 50-ml centrifuge tube.
3. Allow the suspension to settle for 1 h. Pour off and discard the supernatant fluid, and repeat this rinse process at least twice.
4. Decant the saline solution, resuspend the sediment in a small quantity of chlorine-free (spring) water (10 to 20 ml), and pour the suspension into a 500-ml sidearm flask or an Erlenmeyer flask.
5. Add chlorine-free water to the flask so that the fluid level rises to 2 to 3 cm in the side arm or to the top 2 cm of the Erlenmeyer flask. Cover the flask with aluminum foil or black paper, leaving the side arm of the flask exposed to light; if an Erlenmeyer flask is used, cover to 1 cm below the level of fluid in the neck of the flask.
6. Allow the flask to stand at room temperature for several hours or overnight in subdued light.
7. Place a bright light at the side of the flask opposite the surface of exposed water. Do not place the light against the glass, to avoid generation of excess heat. As the eggs hatch, the liberated miracidia will swim to the upper layers and collect in the side arm (or neck region of an Erlenmeyer flask). Make sure that the exposed part of the flask or side arm of the hatching flask does not get too warm.
8. Examine the illuminated area with a magnifying lens (hand lens) to look for minute white organisms swimming rapidly in a straight line (placing dark cardboard or black paper behind the flask will facilitate observation of the white miracidia against a black background).

Results and Patient Reports from Hatching Schistosome Eggs

Living miracidia may be seen; however, failure to see these larvae does not rule out schistosomiasis.

1. Report as "Miracidia of schistosomes detected, indicating the presence of viable eggs."

REVIEW Egg Studies	
Topic	**Comments**
Principle	The specific instances in which information on approximate worm burdens is useful are when one is determining the intensity of infection, deciding on possible chemotherapy, and evaluating the efficacy of the drugs administered. With current therapy, the need for monitoring therapy through egg counts is no longer as relevant. The hatching of schistosome eggs can provide very important information for the physician; if the eggs are viable, treatment is recommended. If nothing but dead eggshells is seen, it is less critical for the patient to receive therapy. **NOTE Both stool and urine should be examined for any potential case of schistosomiasis, regardless of which species is suspected.**
Specimen	Any stool and/or urine specimen that is fresh and has not been refrigerated can be used. For the hatching test or microscopic review for miracidium viability, it is critical that the stool specimen be processed (rinsed, centrifuged) with 0.85% NaCl rather than water, which would stimulate the eggs to hatch prematurely.
Examination	Multiple egg counts should be performed on the same specimen; it is also recommended that clinical decisions be based on a series of egg counts over time, particularly when one is monitoring therapy. All schistosome eggs recovered (but not hatched) should be examined under the microscope for moving cilia (flame cells) within the miracidium larvae.
Results and Laboratory Reports	The failure to recover nematode eggs does not completely rule out the possibility of infection; however, the probability of infection is very low when results are negative, particularly with helminths such as *T. trichiura*, *A. lumbricoides*, and hookworm. When schistosome eggs are reported, the viability of the eggs (miracidium larva within the eggshell) must be indicated. If dead eggshells are seen, the "nonviability" of these eggs also needs to be indicated.
Procedure Notes and Limitations	Egg counts are always subject to error; multiple counts are recommended. When performing egg counts, remember to use the stool consistency factors when calculating the total number of eggs. The failure to find schistosome eggs or to see evidence of egg viability does not rule out the possibility of schistosomiasis, particularly when a long-standing infection is suspected.

2. Report as "No miracidia of schistosomes detected; presence of eggs is not ruled out by this procedure."

Procedure Notes for Hatching Schistosome Eggs

1. Both urine and stool specimens must be collected without preservatives and should not be refrigerated before being processed. Regardless of the species suspected, **BOTH URINE AND STOOL SHOULD BE EXAMINED FOR EVERY PATIENT WITH POTENTIAL SCHISTOSOMIASIS.**
2. Hatching does not occur until the saline is removed and nonchlorinated water is added. If a stool concentration is performed, use saline throughout the procedure to prevent premature hatching.
3. Make sure that the light is not too close to the side arm or top layer of water in the Erlenmeyer flask. Excess heat kills the miracidia.

Procedure Limitations for Hatching Schistosome Eggs

1. The absence of live miracidia does not rule out the presence of schistosome eggs. Nonviable eggs or eggs that failed to hatch are not detected by this method. Microscopic examination of direct or concentrated specimens should be used to demonstrate the presence or absence of eggs.
2. Egg viability can be determined by placing some stool or urine sediment (same material as used for the hatching flask) on a microscope slide. Low-power magnification (×100) can be used to locate the eggs. Individual eggs can be examined under high dry magnification (×400); the detection of moving cilia on the flame cells (primitive excretory system) confirms egg viability.
3. Free-living ciliates may be present in soil-contaminated water. Therefore, it may be necessary to perform the following steps to differentiate those forms from the parasitic miracidia (26).
   A. Transfer a few drops of the suspension containing the organisms to a 3 x 2-in. slide, and examine under the microscope.
   B. Add a drop of weak iodine solution (weak-tea color) or dilute methylene blue (pale blue).
   C. Parasitic miracidia will stop moving, while free-living forms continue to move.

Search for Tapeworm Scolex

Since the medication used for treatment of tapeworms is usually very effective, a search for tapeworm scolices is rarely requested and no longer clinically relevant. However, stool specimens may have to be examined for the presence of scolices and gravid proglottids of cestodes for proper species identification. This procedure requires mixing a small amount of feces with water and straining the mixture through a series of wire screens (graduated from coarse to fine mesh) to look for scolices and proglottids. *Remember to use standard precautions and wear gloves when performing this procedure.* The appearance of scolices after therapy is an indication of successful treatment. If the scolex has not been passed, it may still be attached to the mucosa; the parasite is capable of producing more segments from the neck region of the scolex, and the infection continues. If this occurs, the patient can be retreated when proglottids begin to reappear in the stool.

After treatment for tapeworm removal, the patient should be instructed to take a saline cathartic and to collect all stool material passed for the next 24 h. The stool material should be immediately placed in 10% formalin, thoroughly broken up, and mixed with the preservative (1-gal [3.8-liter] plastic jars, half full of 10% formalin, are recommended).

For additional information, see chapter 8 (Fixation and Special Preparation of Fecal Parasite Specimens and Arthropods).

Quality Control for the Tapeworm Scolex Search

1. Follow routine procedures for optimal collection and handling of fresh fecal specimens for parasitologic examination.
2. Review diagrams and sizes of proglottids and scolices of tapeworms.

Procedure for the Tapeworm Scolex Search

1. Mix a 24-h stool specimen (fresh or preserved in 10% formalin) with water, and thoroughly break up the specimen to make a watery suspension.
2. Strain the suspension (or the purged stool) through a double layer of screen wire or a sieve (a coarse-mesh screen placed over a fine-mesh screen).
3. Wash off the sediment remaining from each portion by running a slow flow of tap water over it.
4. Examine the cleansed debris with a hand lens to look for scolices and proglottids (the *Taenia* scolex is 0.5 to 1 cm long and 1 to 2 mm wide).
5. Repeat steps 3 and 4 for each portion of the suspension strained.
6. Collect the strained sediment in a glass petri dish, and place the dish over a black surface to increase the contrast of organisms against the background.
7. Observe with a magnifying hand lens, and pick pieces of worms with an applicator stick.
8. Rinse gravid proglottids and/or scolices with tap water, blot them dry on paper towels, and place them between two microscope slides separated at the edges by thin pieces of cardboard.

REVIEW Search for Tapeworm Scolex

Topic	Comments
Principle	The procedure requires mixing feces (from a 24-h stool specimen preserved with 10% formalin) with water and straining the mixture through a series of wire screens, graduated from coarse to fine mesh, to look for scolices and proglottids. The same procedure may be used to look for small adult nematodes and trematodes. Appearance of scolices after therapy is an indication of successful treatment. However, with effective therapies available, the need for this procedure has become relatively rare.
Specimen	Any posttherapy 24-h stool specimen submitted in 10% formalin, preferably collected after the patient has taken a saline cathartic, can be used. Single stools in 10% formalin can be submitted, but the chance of finding the scolex is minimal.
Examination	A hand lens may be necessary during the search for the scolex. Also, remember to place the material in the screen over something black to provide more contrast between the dark background and the pale yellow to white scolex. The entire specimen must be strained and examined before negative results are reported.
Results and Laboratory Reports	If the scolex or proglottids are recovered, they should be identified to species level if possible, particularly if the tapeworm is *T. solium*.
Procedure Notes and Limitations	Remember that the eggs of *T. solium* (pork tapeworm) are infectious for humans (cysticercosis); *all stools should be handled carefully, and gloves should be worn at all times. T. solium* eggs within the uterine branches of a gravid proglottid may not be preserved, even in 10% formalin. Failure to find the tapeworm scolex does not rule out the possibility of cure, particularly when a saline cathartic has not been used.

9. Fasten the preparation by placing rubber bands at each end of the slides so that the segments become somewhat flattened. One can also perform the India ink injection of the proglottid (see next protocol below).
10. Observe under the low power of a dissecting microscope for the number of uterine branches and genital pores in the segments and the presence or absence of a rostellum of hooks on the scolex. Quite often, the scolex is broken off from the rest of the strobila (chain of proglottids) and is ~1 cm long.

Results and Patient Report from the Tapeworm Scolex Search

Tapeworm proglottids may be recovered (either singly or several attached together), and a scolex may or may not be seen.

1. Report as "A search for adult worms reveals the presence/absence of . . . (finding)."
   Example: *Taenia saginata* scolex present

Procedure Notes for the Tapeworm Scolex Search

1. Remember that *Taenia solium* eggs are infective (cysticercosis), as are the eggs of *Hymenolepis nana*.
2. *Wear gloves when performing this procedure.*
3. Specimens preserved in 10% formalin are recommended.
4. If the patient has received niclosamide or praziquantel, a purged specimen is required, which should be immediately preserved in 10% formalin.
5. Wood's lamp may be used to reveal the scolices if the patient has been given quinacrine dyes; the worms will have absorbed the dye, and they fluoresce at a wavelength of 360 nm. Also, after the use of quinacrine, tapeworm proglottids appear yellow.

Procedure Limitations for the Tapeworm Scolex Search

1. Niclosamide or praziquantel therapy leads to dissolution of the tapeworm. Therefore, the scolex and other proglottids may be difficult to recover unless the patient receives a saline purge soon after taking the medication.
2. It is often difficult to identify proglottids without staining. Identification may be achieved by staining with India ink.

India Ink Injection Procedure for Tapeworm Proglottids

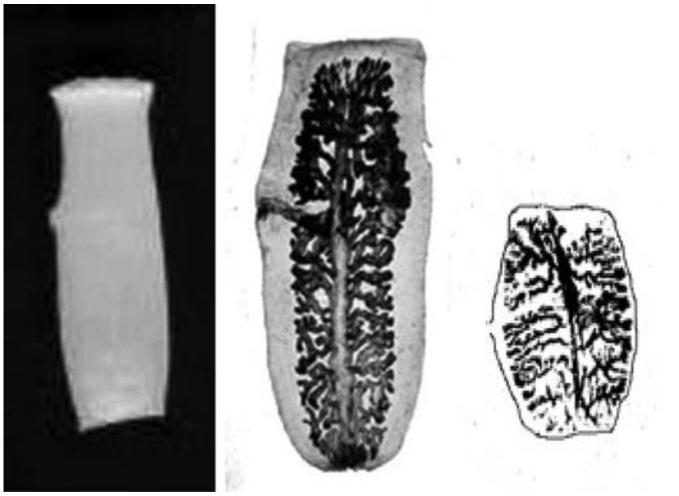
Identification of adult worms usually involves examination of a tapeworm proglottid. A *Taenia* proglottid must be gravid, containing the fully developed uterine branches. Using a syringe (1 ml or less) and a 25-gauge needle, India ink is injected into the central uterine stem of the proglottid, filling the uterine branches with ink, or into the uterine pore. The proglottid can then be rinsed in water or saline, blotted dry on paper towels, pressed between two slides, and examined. After the identification has been made, the proglottid can be left between the two slides (place a rubber band around the slides), dehydrated through several changes of ethyl alcohol (50, 70, 90, and 100%), cleared in two changes of xylene, and mounted with Permount for a permanent record. After the xylene step, the proglottid will be stuck to one of the two slides; do not try to remove it (it is very brittle and will crack) but merely add the Permount to the proglottid and then add the coverglass (Fig. 4.9).

It is important to remember that even when submitted in fixative, the tapeworm eggs within the branched uterine structure may not be killed and may remain infective. Since this procedure is usually limited to tapeworms in the genus *Taenia*, if eggs of *Taenia solium* (pork tapeworm) are present, they present a potential danger in terms of possible egg ingestion/inhalation/swallowing leading to cysticercosis. This becomes a possibility when performing the actual ink injection. If the needle accidentally pokes through the tapeworm proglottid, an aerosol can be created that may contain infectious eggs. **ONE SHOULD WEAR A MASK, GLOVES, AND LABORATORY COAT AT ALL TIMES WHEN PERFORMING THIS PROCEDURE.**

Procedure for the Tapeworm India Ink Injection (MASK, LABORATORY COAT, AND GLOVES MUST BE WORN AT ALL TIMES WHEN PERFORMING THIS PROCEDURE)

1. Using a 1-ml syringe and a 25-gauge needle, fill the syringe with approximately 0.5 ml of India ink.
2. Rinse the proglottid with tap water, and blot it dry on paper towels.

Figure 4.9 *Taenia* gravid proglottids after India ink injection of the uterine branches. (Left) *Taenia* fresh gravid proglottid prior to India ink injection. (Middle) *Taenia saginata* gravid proglottid. (Right) *Taenia solium* gravid proglottid.
doi:10.1128/9781555819002.ch4.f9



3. To prepare for the injection, hold the proglottid between your thumb and first finger or on the surface of the counter (harder to do).
5. Very carefully, penetrate either the central uterine stem at the end where the proglottid has become unattached from the chain or penetrate the side uterine pore with the end of the needle.
6. GENTLY inject some India ink; it should penetrate the uterine branches very quickly. IF THE INJECTION DOES NOT IMMEDIATELY OCCUR, DO NOT FORCE THE INJECTION.
7. You can try the other entry location, again GENTLY injecting the ink.
8. After the injection, rinse the gravid proglottid with tap water, blot dry on paper towels, and place the proglottid between two microscope slides separated at the edges by thin pieces of cardboard.
9. Fasten the preparation by placing rubber bands at each end of the slides so that the segments become somewhat flattened.
10. Observe the number of uterine branches on ONE SIDE ONLY where they come off the main uterine stem; generally *Taenia saginata* has 12 or more branches, while *Taenia solium* has approximately 8 or fewer branches.
11. After the identification has been made, the proglottid can be left between the two slides (place a rubber band around the slides), dehydrated through several changes of ethyl alcohol (50, 70, 90, and 100%), cleared in two changes of xylene, and mounted with Permount for a permanent record.
12. After the xylene step, the proglottid will be stuck to one of the two slides; do not try to remove it (it is very brittle and will crack) but merely add the Permount to the proglottid and then add the coverglass.

Results and Patient Report from the India Ink Injection Procedure

Tapeworm proglottids may be recovered (either singly or several attached together).

1. Report as:
   Example: *Taenia saginata* gravid proglottid present

Procedure Notes for the India Ink Injection Procedure

1. Remember that *T. solium* eggs are infective (cysticercosis). The identification to the species level may not be possible until after the ink injection is completed.
2. *Wear mask, laboratory coat, and gloves when performing this procedure.*

3. Fresh, unpreserved proglottids are recommended; preserved proglottids can be used, but they are harder to inject.
4. Make sure the proglottid is blotted dry prior to the injection; otherwise, it may be difficult to hold onto the proglottid—it can slip through your fingers.
5. DO NOT FORCE THE INK INJECTION. If the ink does not easily flow into the branches, it may be because the proglottid is **not gravid, but only mature.** Also, the needle may not be in the right place.
6. IF THE INK INJECTION IS FORCED, it is more likely you may create an aerosol, thus increasing the danger for exposure to viable *T. solium* eggs.
7. Remember that after the xylene step, the proglottid will be stuck to one of the two slides; do not try to remove it (it is very brittle and will crack) but merely add the Permount to the proglottid and then add the coverglass.

Procedure Limitations for the India Ink Injection Procedure

1. If the proglottid is fresh, it may have begun to disintegrate, thus making the ink injection more difficult.
2. If the proglottid is mature AND NOT YET GRAVID, the branches may not inject properly.
3. If the ink injection is not successful, one can try some of the mounting methods found in chapter 9.

Qualitative Test for Fecal Fat

The microscopic examination of stool with the addition of Sudan III is a very simple, quick, and widely used technique to screen the specimen for fat. Fresh, unpreserved fecal material is required. If there is a time delay prior to testing, the specimen should be refrigerated. Specimens collected more than 48 h earlier or specimens that are dried out should be discarded, and fresh specimens should be collected. Although this technique is quite old and the original paper may be difficult to obtain, it was published in 1961 (32).

Sudan Black IV Stain, also known as scarlet red, was introduced by Michaelis in 1901 as a fat stain. It is a dimethyl derivative of Sudan III, which makes it a deeper and more intense stain, but it has similar physical properties and is fat soluble. This stain has been widely used as a screening method because it is easy to use and correlates well with quantitative methods.

Sudan Black IV Stain is used as a qualitative method to detect the presence of fecal fat. Normally the stool does not contain more than 20 g of fat daily. In patients with steatorrhea, fat malabsorption occurs, and greater quantities of fat are detected in the stool. This procedure, when performed carefully and consistently, is a simple method of detecting this condition in the patient (33). Other dyes such as Sudan II, Sudan III, Oil Red O, and Sudan Black B can be used as well.

Sudan Stain

95% ethanol	
Glacial acetic acid (36% [vol/vol])	
Sudan III stain	
or	
Sudan Black IV, certified (Hardy Diagnostics, Santa Maria, CA)	3.0 mg
95% ethanol	740 ml
Deionized water	260 ml

Quality Control for the Qualitative Test for Fecal Fat

1. Follow routine procedures for optimal collection and handling of fresh fecal specimens for parasitology.
2. Run a QC sample with each batch of patient tests as in procedure below.
3. As a positive control, mayonnaise is used; red-stained fat globules should be observed microscopically.
4. As a negative control, water is used; no fat globules are observed.
5. Record all QC results. If the QC results are unacceptable, the test must be repeated and documented on the corrective action sheet.

Procedure for the Qualitative Test for Fecal Fat (Sudan III)

1. Mix a small amount of stool with an equal amount of 95% ethanol in a test tube. Mix well.
2. Add 2 drops of Sudan III stain to the stool-ethanol mixture. Mix well, and let stand for a few minutes.
3. Using a pipette, remove a drop from the test tube and place it on a slide. Cover with a coverslip.
4. Using the microscope, examine the slide for globules of fat stained red (neutral fat) and needle-like crystals (fatty acid).
5. Add several drops of glacial acetic acid to the test tube. Remove a drop from the test tube and place it on a slide. Cover with a coverslip.
   A. Gently heat the slide over a flame. (Do not boil.)
   B. Observe again on the microscope for globules of fat stained red (Fig. 4.10).

Results and Patient Reports for the Qualitative Test for Fecal Fat (Sudan III)

Red-stained fat globules or fatty acid crystals are seen microscopically. Report the specimen as "fat not increased" or "fat increased" depending on the number of globules seen.

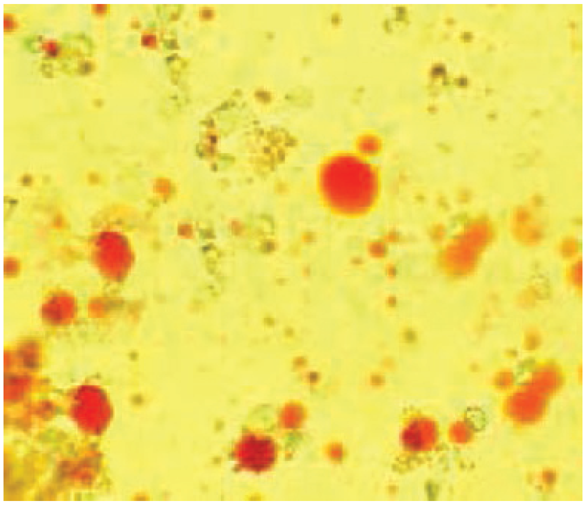


Figure 4.10 Fat droplets seen in the fecal fat test. The color can range from red to orange (pale to more intense), and the droplets can vary in size. doi:10.1128/9781555819002.ch4.f10

1. If fewer than 100 globules/high-power field (hpf) are seen before or after heating, report as “fat not increased.”
2. If more than 100 globules/hpf of fat are seen before or after heating, report as “fat increased.”
3. If fatty acid crystals have been seen before heating and globules appear after heating, report as “fatty acids increased.”

Procedure for the Qualitative Test for Fecal Fat (Sudan Black IV)

1. Place a small aliquot of stool suspension on a clean glass slide.
2. Mix 2 drops of 95% ethanol with the suspension on the slide.
3. Add 2 drops of Sudan Black IV Stain to the suspension on the slide, and mix well.
4. Cover the suspension with a coverslip, and examine microscopically for the presence of large orange or red droplets.

Results and Patient Reports for the Qualitative Test for Fecal Fat (Sudan Black IV)

1. Fatty acids are present as lightly staining flakes or needle-like crystals that do not stain.
2. Soaps appear as nonstaining formless flakes, coarse crystals, or rounded masses.
3. Neutral fats appear as large orange or red droplets. If 60 or more stained droplets (neutral fats) are seen per 40× (high-power) field, it is a presumptive finding that the patient has steatorrhea.

Procedure Limitations for the Qualitative Test for Fecal Fat

1. The formation of large needle-like crystals as the preparation cools after heating does not necessarily mean that the original globules were fatty acid. Sudan III forms very short needle-like crystals in bunches as it dries.
2. Very few, if any, neutral fat globules are seen in a normal stool specimen. The presence of large amounts of neutral fat should raise suspicion that the patient has ingested mineral oil or castor oil, thus causing a false-positive result.
3. Do not count the fat that is present in vegetable cells.

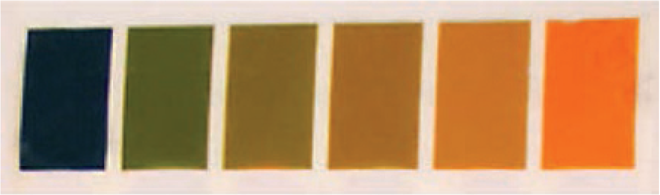
Quantitation of Reducing Substances (Clinitest)

Clinitest is a reagent tablet test based on the classic Benedict’s copper sulfate reduction reaction, combining ingredients with an integral heat-generating system. Clinitest provides clinically useful information about carbohydrate metabolism by determining the amount of reducing substance in urine or stool. Reducing substances convert the cupric sulfate ($CuSO_4$) to cuprous (Cu_2O) oxide and cause a change in solution color ranging from blue through green to orange (Fig. 4.11). Unpreserved stool is required; the specimen should be placed in the refrigerator if there is a delay in testing. Specimens collected more than 48 h earlier or specimens that are dried out should be discarded, as fresh specimens should be collected. Although this is a relatively old method, there are references in the literature (34).

Clinitest Tablets

Clinitest reagent tablets (store tablets at room temperature in a plastic bag)
Comparative color chart that comes with the tablets
Deionized water
Chek-Stix positive control

Figure 4.11 Color range for stool Clinitest reactions: blue (negative) to orange (positive). doi:10.1128/9781555819002.ch4.f11



Quality Control for Quantitation of Reducing Substances (Clinitest)

1. Follow routine procedures for optimal collection and handling of fresh fecal specimens for parasitology.
2. Run a QC sample with each batch of patient tests as in procedure below.
3. As a positive control, Chek-Stix is used; the development of a green, yellow, or orange color with a yellow or red precipitate is considered a positive result.
4. As a negative control, 0.5 ml of deionized water is used; blue color is considered a negative result.
5. Record all QC results. If the QC results are unacceptable, the test must be repeated and documented on the corrective action sheet.

Procedure for Quantitation of Reducing Substances (Clinitest)

1. All testing on clinical specimens should be performed in a biological safety cabinet by personnel wearing gloves and a laboratory coat.
2. Add 1 volume of stool to 2 volumes of deionized water, and mix thoroughly.
3. Using a disposable transfer pipette, transfer 15 drops of this suspension into a clean test tube.
4. Drop one Clinitest tablet reagent into the test tube.
5. Observe the reaction. Do not shake the tube while the chemical reaction is occurring.
6. Wait 15 s after the reaction stops, then gently shake contents to mix.
7. Compare the color of the liquid to the color chart in the package insert of the Clinitest tablet reagent (Fig. 4.11).
8. Discard supplies in appropriate biohazard containers.

Results and Patient Reports for Quantitation of Reducing Substances (Clinitest)

1. Negative: clear to cloudy blue color
2. Positive: compare the liquid color to the color chart that comes with the tablets, and grade the degree of color development to the color chart (trace, 1+, 2+, 3+, or 4+). These results equate to the grams per deciliter of the reducing substance present per sample. The colors range from blue through green through yellow/orange to orange (negative to 4+).
3. Positive: report as Trace (0.25 g/dl), 1+ (0.5 g/dl), 2+ (0.75 g/dl), 3+ (1.0 g/dl), or 4+ (equal to or greater than 2 g/dl)
   Example: 1+ (0.5 g/dl)
4. Negative
   Example: Negative

Procedure Limitations for Quantitation of Reducing Substances (Clinitest)

1. Clinitest is not specific for glucose and reacts with any reducing substance in stool, including lactose, fructose, galactose, and pentoses.
2. Interfering substances may affect the results. These include salicylates, penicillin, large quantities of ascorbic acid, nalidixic acid, and cephalosporins.
3. Failure to observe the reaction at all times may lead to erroneously low results if reducing substances are present in large amounts. If more than 2% sugar is present, a rapid color change may occur during boiling, causing the color to pass rapidly through bright orange to a dark brown or greenish brown.

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5

Examination of Other Specimens from the Intestinal Tract and the Urogenital System

Examination for pinworm
Cellulose tape preparations
Anal swabs
Sigmoidoscopy material
Direct saline mount
Permanent stained slide
Duodenal contents
Duodenal drainage
Duodenal capsule technique (Entero-Test)
Urogenital specimens
Trichomoniasis
Filariasis
Schistosomiasis

Examination for Pinworm

Enterobius vermicularis, known as the pinworm or seatworm, is a roundworm parasite that has worldwide distribution and is commonly found in children. The adult female worm migrates out of the anus, usually at night, and deposits her eggs on the perianal area. The adult female (8 to 13 mm long) is occasionally found on the surface of a stool specimen or on the perianal skin. Since the eggs are usually deposited around the anus, they are not commonly found in feces and must be detected by other diagnostic techniques. Diagnosis of pinworm infection is usually based on the recovery of typical eggs, which are described as thick-shelled, football-shaped eggs with one slightly flattened side. Each egg often contains a fully developed embryo and will be infective within a few hours after being deposited.

The most striking symptom of this infection is pruritus, which is caused by the migration of the female worms from the anus onto the perianal skin before egg deposition. The sometimes intense itching results in scratching and occasional scarification. In most infected people, this may be the only symptom, and many individuals remain asymptomatic. Eosinophilia may or may not be present.

Infections tend to be more common in children and occur more often in females than in males. In heavily infected females, there may be a mucoid vaginal discharge, with subsequent migration of the worms into the vagina, uterus, fallopian tubes, appendix, or other body sites including the urinary tract, where they become encapsulated (1–8). Although tissue invasion has been attributed to the pinworm, these cases are not numerous. Symptoms that have been attributed to the pinworm infection, particularly in children, include nervousness, insomnia, nightmares, and even convulsions. In some cases, perianal granulomas may result (3).

In one report, a homosexual man presented with severe abdominal pain and hemorrhagic colitis, eosinophilic inflammation of the ileum and colon, and numerous unidentifiable larval nematodes in the stool. Using morphologic characteristics and molecular cloning of nematode rRNA genes, the parasites were identified as larvae of *E. vermicularis*; these larvae are rarely seen and are not thought to cause disease. The authors stated that occult enterobiasis is widely prevalent and may be a cause of unexplained eosinophilic enterocolitis (9).

doi:10.1128/9781555819002.ch5

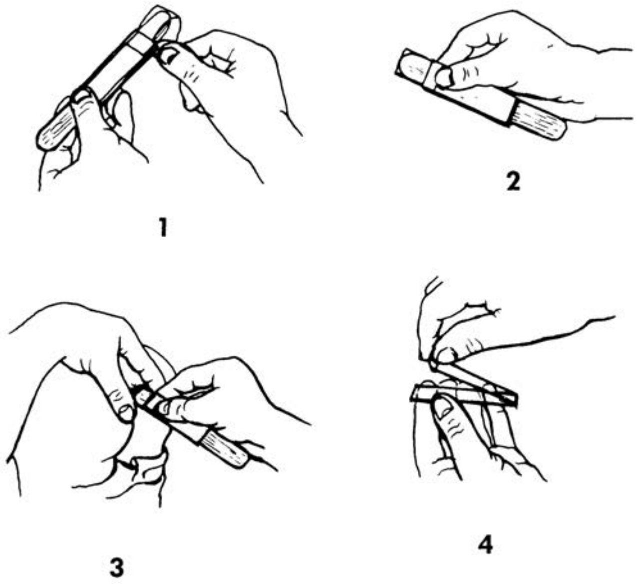
Cellulose Tape Preparations

The most widely used diagnostic procedure for pinworm infection is the cellulose tape (adhesive cellophane tape) method (5, 10–14) (Fig. 5.1 to 5.3). Several commercial collection procedures are also available. Specimens should be obtained in the morning before the patient bathes or goes to the bathroom. At least four to six consecutive negative slides should be observed before the patient is considered free of infection. Occasionally adult female pinworms are seen on the tapes or swabs.

Collection of the Specimen

1. Place a strip of cellulose tape on a microscope slide, starting 1/2 in. (1 in. = 2.54 cm) from one end and running toward the same end, continuing around this end lengthwise; tear off the strip flush with the other end of the slide. Place a strip of paper, 1/2 by 1 in., between the slide and the tape at the end where the tape is torn flush.
2. To obtain the sample from the perianal area, peel back the tape by gripping the label, and with the tape looped (adhesive side outward) over a wooden tongue depressor held against the slide and extended about 1 in. beyond it, press the tape firmly against the right and left perianal folds.
3. Spread the tape back on the slide, adhesive side down.
4. Write the name and date on the label.

Figure 5.1 Collection of *Enterobius vermicularis* eggs by the cellulose tape method. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch5.f1



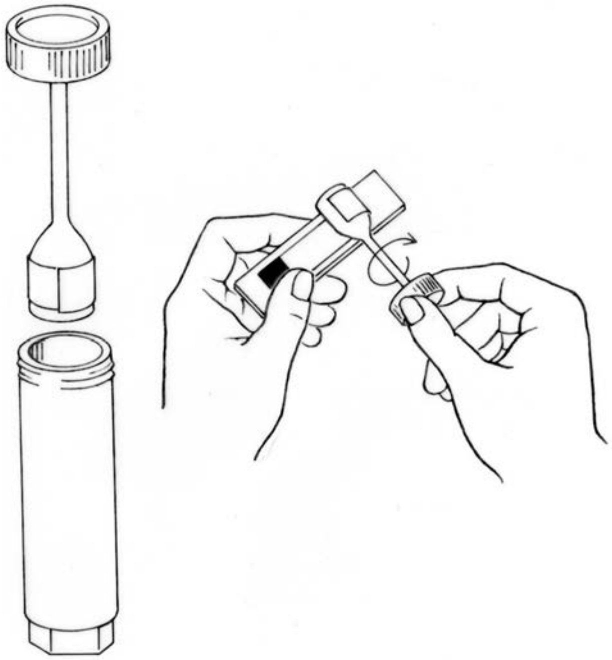


Figure 5.2 Diagram of a commercial kit (Evergreen Scientific) for use in sampling the perianal area for the presence of pinworm (*E. vermicularis*) eggs. On the left is the vial containing the sampler, which has sticky tape around the end. Once this is applied to the perianal area and eggs are picked up on the tape, the label area is placed at one end of the slide. The sticky tape is rolled down the slide and attaches to the glass. This device is easy to use and provides an area sufficient for adequate sampling. A minimum of four to six consecutive negative tapes are required to rule out a pinworm infection; most laboratories are accepting four rather than requesting the full six. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch5.f2

Note Do *not* use Magic transparent tape; use regular clear cellulose tape. If Magic tape is submitted, a drop of immersion oil can be placed on top of the tape to facilitate clearing.

Examination

Lift one side of the tape, apply 1 small drop of toluene or xylene, and press the tape down on the glass slide. The preparation will then be cleared, and the eggs will be visible. Examine the slide with low power and low illumination.

Anal Swabs

The anal swab technique (15) is also available for the detection of pinworm infections; however, most laboratories use the cellulose tape method because it eliminates the necessity for preparing and storing swabs. At least four to six consecutive negative swabs should be obtained before the patient is considered free of infection.

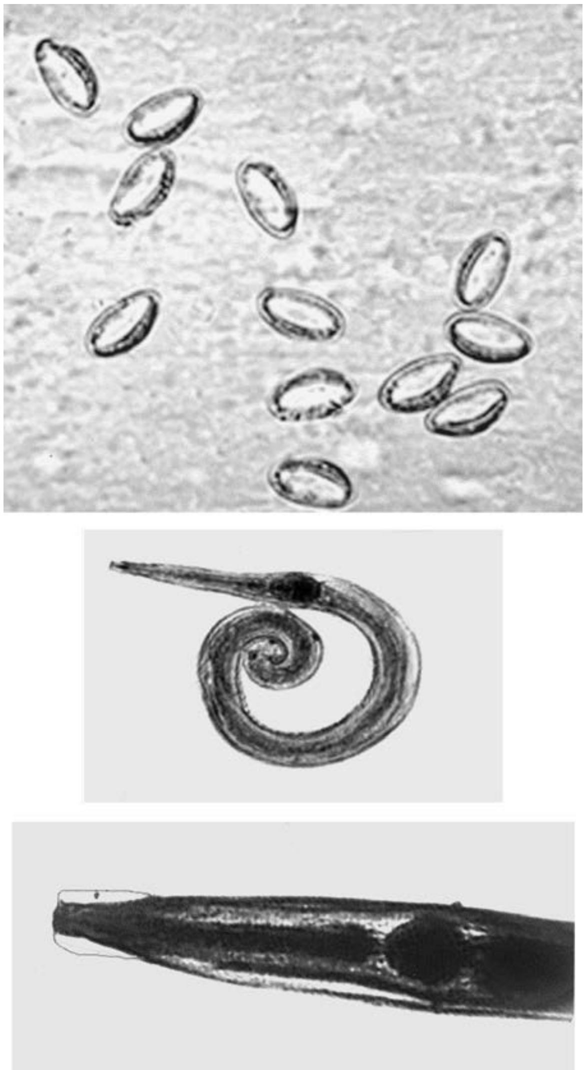


Figure 5.3 (Top) *Enterobius vermicularis* (pinworm) eggs seen in a Scotch tape preparation; note the football-shaped eggs with one side a bit flatter than the other. In some preparations, eggs are seen that contain fully developed larvae; such eggs are infective. (Middle) Adult female pinworm found on a collection device. Note the large, round esophageal bulb. (Bottom) Adult female pinworm, enlarged anterior end. Note the cephalic expansions around the end of the head, as well as the large, round esophageal bulb. doi:10.1128/9781555819002.ch5.f3

Collection of the Specimen

Specimens should be obtained in the morning before the patient bathes or goes to the bathroom.

1. Prepare a mixture of 4 parts petrolatum to 1 part paraffin, and heat until liquid (just melted).
2. Dip the end of the cotton swab into the mixture, remove the swab, and allow it to cool. If the cotton is not thoroughly coated, dip it again.
3. Store the coated swab in a 100- by 13-mm tube, and plug the end of the tube with cotton. These tubes may be stored for long periods, preferably under refrigeration.
4. Rub the swab gently over the perianal surface and into the folds. Insert the swab into the anal opening about 1/4 in. and then replace it in the tube.

Examination

1. Fill the tube containing the swab half full of xylene or xylene substitute, and let it stand for 3 to 5 min.
2. Remove the swab, and centrifuge the tube at 500 × *g* for 1 min.
3. Remove the supernatant fluid with a pipette (do not pour it off).
4. Place the sediment on a slide, and examine the material for eggs. The fluid can be examined under a coverslip, in a depression slide, or in a wax pencil circle drawn on the slide (to prevent the fluid from spreading).

Sigmoidoscopy Material

Material obtained from sigmoidoscopy can be helpful in the diagnosis of amebiasis that has not been detected by routine fecal examinations; however, a series of at least three routine stool examinations for parasites should be performed on each patient before sigmoidoscopy examination is done. Another option would be to use the immunoassay kits that are designed to detect either the *Entamoeba histolytica*/*E. dispar* group/complex or specifically pathogenic *E. histolytica*; fresh or frozen stools are required for these kits.

Material from the mucosal surface should be aspirated or scraped and should not be obtained with cotton-tipped swabs. If swabs are the only method available, a small amount of cotton should be used on the end of the stick and should be wound tightly to prevent absorption of the sigmoidoscopy material into the cotton. At least six representative areas of the mucosa should be sampled and examined (six samples, six slides) (8, 13).

The specimen should be processed immediately. Usually, the amount of material is limited and should be handled properly to ensure the best examination possible. Various methods of examination are available (direct mount, several options for permanent stains). All are recommended; however, depending on the availability of trained personnel, proper fixation fluids, or the amount of specimen obtained, one or two procedures may be used. If the amount of material limits the examination to one procedure, the use of fixative containing polyvinyl alcohol (PVA) is highly recommended.

If the material is to be examined by any of the new immunoassay detection kits (for *Cryptosporidium* spp. or *Giardia lamblia* [*G. duodenalis*, *G. intestinalis*]), 5 or 10%

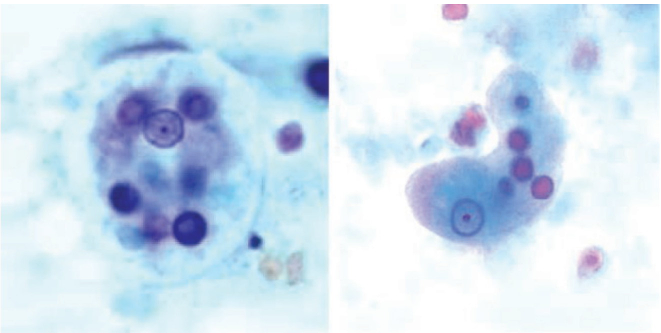
formalin or sodium acetate-acetic acid-formalin (SAF) or the Universal Fixative is recommended. However, as mentioned above, if immunoassay kits are to be used for detection of the *E. histolytica*/*E. dispar* group/complex or specifically pathogenic *E. histolytica*, fresh or frozen stools are required. It is always important to read the package insert to verify collection recommendations. Many physicians performing sigmoidoscopy procedures do not realize the importance of selecting the proper fixative for material to be examined for parasites. For this reason, it is recommended that a parasitology specimen tray (containing Schaudinn's liquid fixative containing PVA, the Universal Fixative, and 5 or 10% formalin) be provided or that a trained technologist be available at the time of sigmoidoscopy to prepare the slides. Even the most thorough examination will be meaningless if the specimen has been improperly prepared.

Direct Saline Mount

If there is no lag time after collection and a microscope is available in the immediate vicinity, some of the material should be examined as a direct saline mount for the presence of motile trophozoites. A drop of material is mixed with a drop of 0.85% sodium chloride and examined under low light intensity for the characteristic movement of amebae. It may take time for the organisms to become acclimated to this type of preparation; therefore, motility may not be obvious for several minutes. There will be epithelial cells, macrophages, and possibly polymorphonuclear leukocytes and red blood cells (RBCs), which will require a careful examination to reveal amebae (Fig. 5.4).

Note Since specific identification of protozoan organisms can be difficult when only the direct saline mount is used, this technique should be used only when sufficient material is left to prepare permanent stained smears.

Figure 5.4 *Entamoeba histolytica* trophozoites stained with trichrome stain. Note the clearly defined internal details (nuclear and cytoplasmic characteristics, plus the ingested red blood cells). doi:10.1128/9781555819002.ch5.f4



Morphologic differentiation between the *E. histolytica*/*E. dispar* group/complex and *Entamoeba coli* can be difficult, in addition to the problem of differentiating human cells from protozoa. Also, unless trophozoites containing ingested RBCs are seen, it is impossible to differentiate organisms in the *E. histolytica*/*E. dispar* group/complex from the actual pathogen, *E. histolytica*.

Permanent Stained Slide

Schaudinn's Fixative

Most of the material obtained at sigmoidoscopy can be smeared (gently) onto a slide and immediately immersed in Schaudinn's fixative. These slides can then be stained with trichrome stain and examined for specific cell morphology, either protozoan or otherwise. The procedure and staining times are identical to those for routine fecal smears.

PVA Fixative

If the material is bloody, contains a lot of mucus, or is a "wet" specimen, a few (no more than 2 or 3) drops of fixative containing PVA can be mixed with 1 or 2 drops of specimen directly on the slide, which is allowed to air dry (a 37°C incubator can be used) for at least 2 h before being stained. If time permits, the PVA smears should be allowed to dry overnight; they can be routinely stained with trichrome stain and examined as a permanent mount.

SAF Fixative

Material obtained at sigmoidoscopy can be placed in small amounts of SAF. After fixation for 30 min, the specimen can be centrifuged for 10 min at 500 × *g*, and smears from the small amount of sediment can be prepared for permanent staining with iron hematoxylin (trichrome stain would be the second choice). One of the organisms most strongly suspected when sigmoidoscopy is performed is *E. histolytica*, whose morphology is normally seen from the permanent stained smear; however, this identification assumes that RBCs are seen within the cytoplasm of the trophozoites (Fig. 5.4). If RBCs are not seen in the trophozoite cytoplasm, the report should indicate that organisms in the *E. histolytica*/*E. dispar* group/complex have been seen and identified. However, if SAF is used, both the permanent stained smear and a fecal immunoassay kit can be used. If enough material for only a single procedure is available, the permanent stained smear is recommended, particularly if the iron hematoxylin stain (incorporating the carbol fuchsin step) is used (34).

Universal Fixative

Material obtained at sigmoidoscopy can be placed in small amounts of the Universal Fixative (TOTAL-FIX). After fixation for 30 min, the specimen can be centrifuged for 10 min at 500 × *g*, and smears from the small amount of sediment can be prepared for permanent staining with trichrome or iron hematoxylin. The sediment should be smeared onto a slide and allowed to dry 30 min at 37°C prior to staining. If the material is thoroughly dry, no albumin or PVA is required. One of the organisms most strongly suspected when sigmoidoscopy is performed is *E. histolytica*, whose morphology is normally seen from the permanent stained smear; however, this identification assumes that RBCs are seen within the cytoplasm of the trophozoites (Fig. 5.4). If RBCs are not seen in the trophozoite cytoplasm, the report should indicate that organisms in the *E. histolytica/E. dispar* group/complex have been seen and identified. However, if the Universal Fixative is used, both the permanent stained smear and some molecular tests or panels can be used. If enough material for only a single procedure is available, the permanent stained smear is recommended.

Duodenal Contents

Duodenal Drainage

In infections with *Giardia*, *Strongyloides*, or *Cryptosporidium*, routine stool examinations may not reveal the organisms. Duodenal drainage material can be submitted for examination as direct or concentration wet mounts or permanent stained smears, techniques that may reveal the parasites (Fig. 5.5). *Use standard precautions and wear gloves when handling the specimen.*

The specimen should be submitted to the laboratory in a tube containing no preservative; the amount may vary from <0.5 ml to several milliliters of fluid. The specimen may be centrifuged (10 min at 500 × *g*) and should be examined immediately as a wet mount for motile organisms (iodine may be added later to facilitate the identification of any organisms present). If the specimen cannot be completely examined within 2 h after it is taken, any remaining material should be preserved in fixatives containing PVA, SAF, or the Universal Fixative. The "falling-leaf" motility often described for *Giardia* trophozoites is rarely seen in fresh, unpreserved preparations. The organisms may be caught in mucus strands, and the movement of the flagella on the *Giardia* trophozoites may be the only subtle motility seen for these flagellates. *Strongyloides* larvae are usually very motile. Remember to keep the light intensity low.

The duodenal fluid may contain mucus; this is where the organisms, particularly *Giardia*, tend to be found. Therefore, centrifugation of the specimen is important, and the sedimented mucus should be examined. Immunoassay detection kits (*Cryptosporidium* or *Giardia*) can also be used with fresh or formalinized material; however, the kits are specifically recommended for actual stool material.

If a presumptive diagnosis of giardiasis is made on the basis of the wet-preparation examination of the fresh specimen, the coverslip can be removed and the specimen

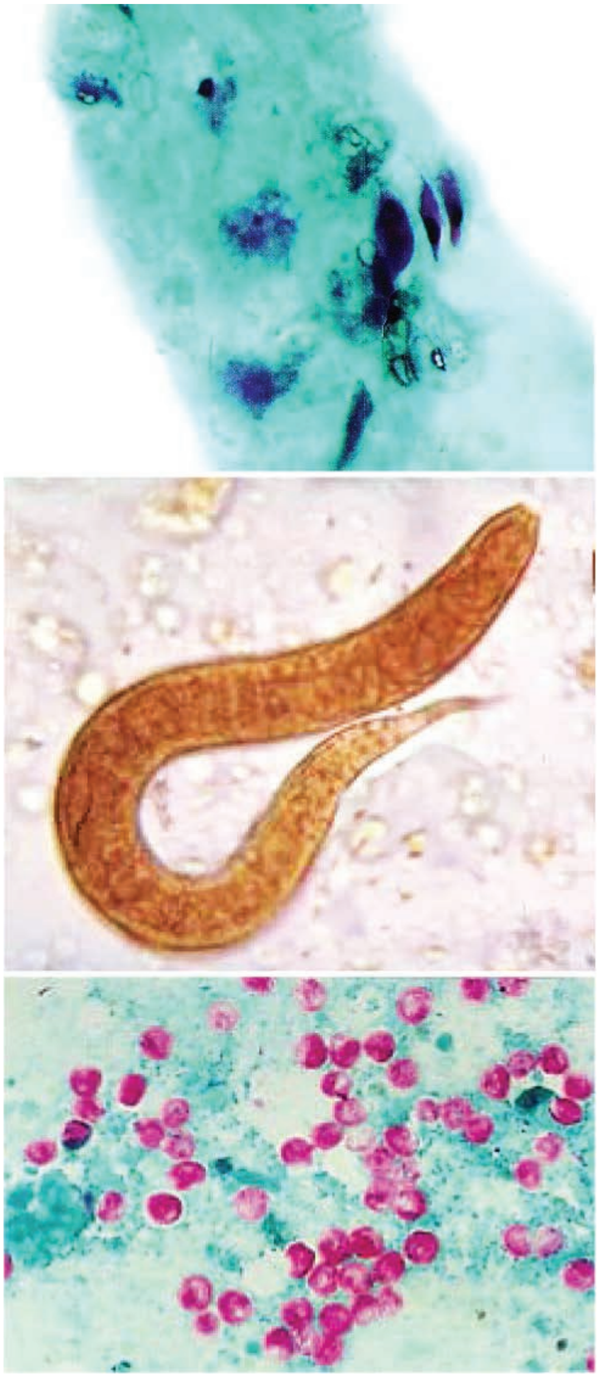


Figure 5.5 Duodenal aspirate specimens. (Top) *Giardia lamblia* in mucus from a duodenal aspirate stained with trichrome stain. Note the teardrop trophozoites (from the front view) and the darker-staining trophozoites that resemble the curved part of a spoon (side view). Also notice the number of organisms contained within the mucus. (Middle) A *Strongyloides stercoralis* rhabditiform larva in a wet mount. The genital primordial packet of cells is visible about halfway down the larva on the left side. (Bottom) *Cryptosporidium* spp. oocysts in duodenal aspirate material stained with a modified acid-fast stain.
doi:10.1128/9781555819002.ch5.f5

can be fixed with one of the fecal fixatives for subsequent staining with either trichrome or iron hematoxylin. If the amount of duodenal material submitted is very small, permanent stains can be prepared rather than using any part of the specimen for a wet-smear examination. Some investigators think that this approach provides a more permanent record, and the potential visual problems with unstained organisms, very minimal motility, and a lower-power examination can be avoided by using oil immersion examination of the stained specimen at ×1,000 magnification.

Duodenal Capsule Technique (Entero-Test)

A simple and convenient method of sampling duodenal contents that eliminates the need for intestinal intubation has been devised (16). The device consists of a length of nylon yarn coiled inside a gelatin capsule (Fig. 5.6). The yarn protrudes through one end of the capsule; this end of the line is taped to the side of the patient's face (Fig. 5.7). The capsule is then swallowed, the gelatin dissolves in the stomach, and the weighted string is carried by peristalsis into the duodenum. The yarn is attached to the weight by a slipping mechanism; the weight is released and passes out in the stool when the line is retrieved after a period of 4 h. Bile-stained mucus clinging to the yarn is then scraped off (mucus can also be removed by pulling the yarn between thumb and finger) and collected in a small petri dish; *disposable gloves should be worn.* Usually 4 or 5 drops of material are obtained.

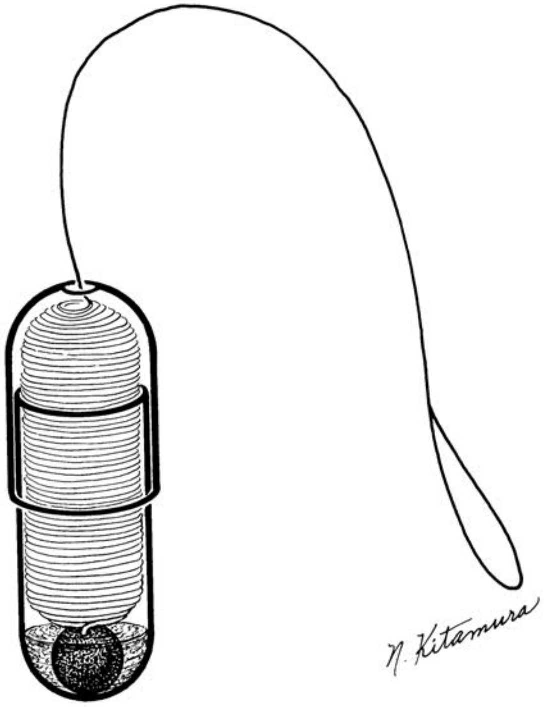


Figure 5.6 Entero-Test capsule for sampling duodenal contents (HCD; Nutri-Link Ltd., Newton Abbot, United Kingdom; adult and pediatric capsules). The device consists of a length of nylon yarn coiled inside a gelatin capsule. The yarn protrudes through one end of the capsule; this end of the line is taped to the side of the patient's face. The capsule is then swallowed, the gelatin dissolves in the stomach, and the weighted string is carried by peristalsis into the duodenum. (Illustration by Nobuko Kitamura.) doi:10.1128/9781555819002.ch5.f6

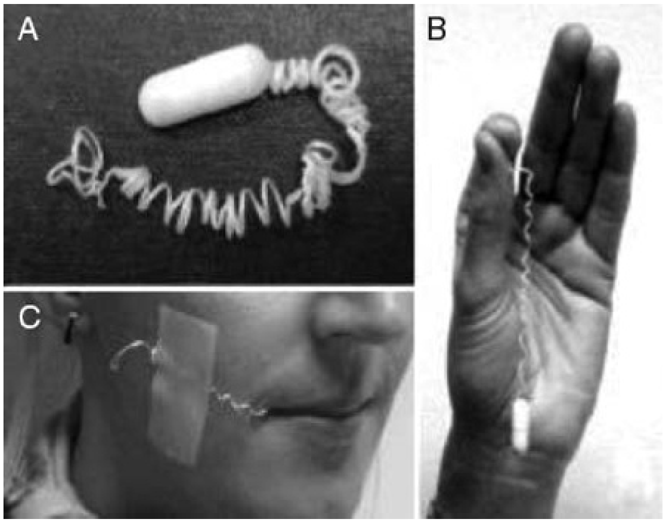


Figure 5.7 Entero-Test string test for sampling duodenal contents. (A) Capsule with partially extracted string; (B) capsule with string before being swallowed; (C) end of the string taped to the cheek. (From Leodolter A et al, *World J Gastroenterol* **11:**584–586, 2005.) doi:10.1128/9781555819002.ch5.f7

The specimen should be examined immediately as a wet mount for motile organisms (iodine may be added later to facilitate the identification of any organisms present). If the specimen cannot be completely examined within an hour after the yarn has been removed, the material should be preserved in one of the fecal fixatives for subsequent staining. Organism motility is like that described above for duodenal drainage.

Note The pH of the terminal end of the yarn should be checked to ensure adequate passage into the duodenum (a very low pH means that it never left the stomach). The terminal end of the yarn should be a yellow-green color, indicating that it was in the duodenum (the bile duct drains into the intestine at this point).

Urogenital Specimens

Trichomoniasis

The identification of *Trichomonas vaginalis* (Fig. 5.8) is usually based on the examination of a wet preparation of vaginal and urethral discharges and prostatic secretions or urine sediment. Multiple specimens may have to be examined before the organisms are detected. These specimens are diluted with a drop of saline and examined under low power and reduced illumination for the presence of actively motile organisms; as the jerky motility begins to diminish, it may be possible to observe the undulating membrane, particularly under high dry power. Recent data indicate that microscopic examination of a spun urine specimen performed in conjunction with microscopic examination of a vaginal fluid specimen improves the detection rate of *T. vaginalis*. While 73% of infections were detected by examination of vaginal fluid specimens and 64% were detected by examination of spun urine, the combined percentage using both methods was 85% (17). It is also important to remember that in older men with nongonococcal urethritis, diagnostic evaluation, empirical treatment, and partner management should include the possibility of infection with *T. vaginalis* (18).

Stained smears are usually not necessary for the identification of this organism. The large number of false-positive and false-negative results reported on the basis of stained smears strongly suggests the value of confirmation by observation of motile organisms from the direct mount, from appropriate culture media (19–22), or from molecular testing (23–25). The ability of Amies gel agar transport medium to maintain the viability of *T. vaginalis* was examined by comparison with specimens immediately inoculated into culture medium. The immediate-inoculation method detected infections in 64 (94.1%) of 68 patients, while the transport method detected infections in 62 (91.2%) of 68 patients (26). However, the data depend on the type of medium used, the time taken for transport, and the temperature at which transport was carried out. Additional information on culture options can be found in chapter 7.

T. vaginalis infection is the most prevalent sexually transmitted disease in the world. To improve diagnostic results, molecular testing is now being used for the detection of this infection and reports excellent results (27–30). There are several FDA-approved molecular diagnostic tests for this organism. They include the APTIMA *Trichomonas vaginalis* Assay (GenProbe, San Diego, CA). This assay utilizes the same amplification technology that has been used for years to detect *Neisseria gonorrheae* and *Chlamydia trachomatis*. It also uses the same specimen types (i.e., clinician-collected vaginal and endocervical swabs, female urine, and PreservCyt solution). It has reported high sensitivity and specificity, and does not require organism viability or motility. It, as well as other antigen-based assays, represents advances in the detection of this parasite.

The Affrirm VPIII DNA probe technology (Becton, Dickinson and Co., Franklin Lakes, NJ) offers a dependable, rapid means for the early identification of three organ-

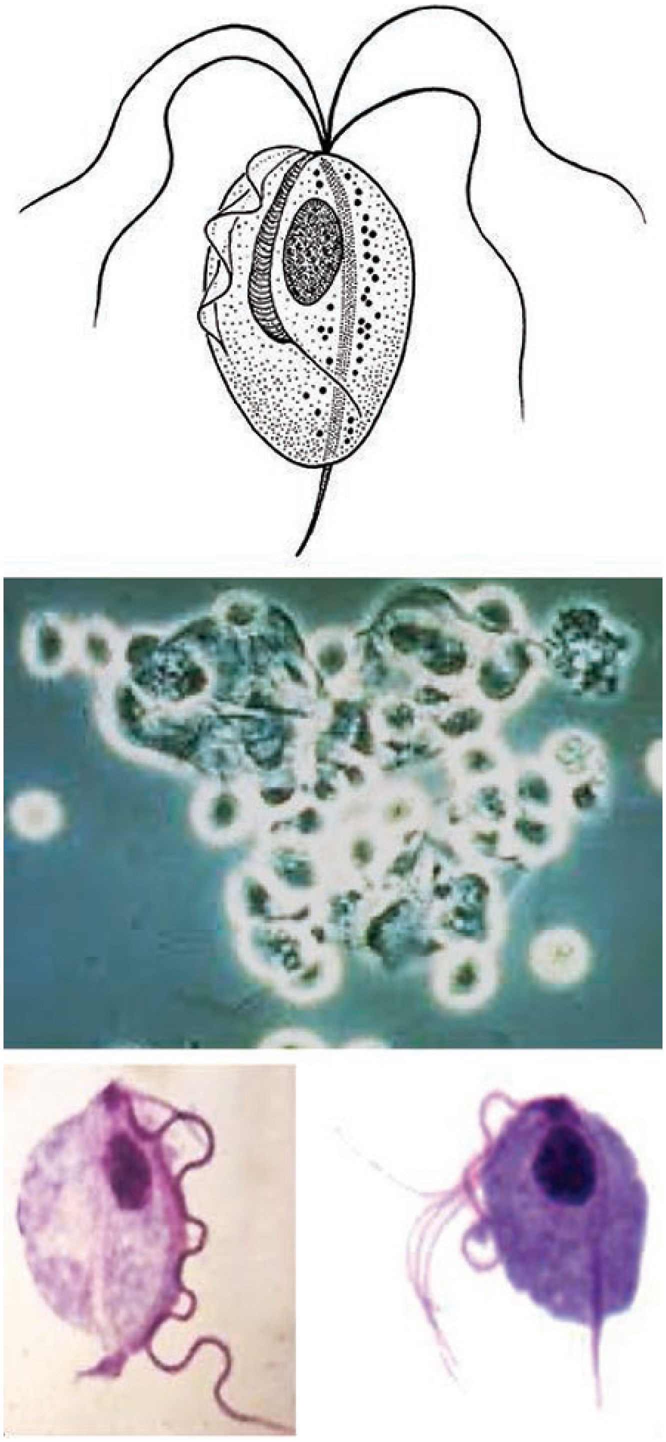


Figure 5.8 (Top) *Trichomonas vaginalis* trophozoite. (Illustration by Sharon Belkin.) (Middle) *T. vaginalis* trophozoites seen in a wet mount preparation. (Bottom, left) *Pentatrichomonas hominis* (stool); (right) *Trichomonas vaginalis* (urinary-genital tract). doi:10.1128/9781555819002.ch5.f8

isms causing vaginitis: *Candida* spp., *Gardnerella vaginalis*, and *T. vaginalis*. During the test protocol, the assay aligns complementary nucleic acid strands to form specific, double-stranded complexes called hybrids. For each organism, this hybrid is composed of capture and color development single-stranded nucleic acid probes, complementary to the released target nucleic acid analyte. Enzyme conjugate then binds to the captured analyte.

Filariasis

Examination of urinary sediment is indicated in certain filarial infections. The occurrence of microfilariae in urine has been reported with increasing frequency in *Onchocerca volvulus* infections in Africa. The triple-concentration technique is recommended for the recovery of microfilariae (13) (see chapter 7).

Urine is collected into a bottle, the volume is recorded, and thimerosal (1 ml/100 ml of urine) is added. The specimen is placed in a funnel fitted with tubing and a clamp; this preparation is allowed to settle overnight. The following day, 10 to 20 ml of urine is withdrawn and centrifuged. The supernatant fluid is discarded, and the sediment is resuspended in 0.85% NaCl. This preparation is again centrifuged, and 0.5 to 1.0 ml of the sediment is examined under the microscope for the presence of nonmotile microfilariae. The membrane filtration technique used with blood can also be used with urine for the recovery of microfilariae. Administration of the drug diethylcarbamazine (Hetrazan) has been reported to enhance the recovery of microfilariae from the urine (31).

Figure 5.9 Membrane filtration system. (Upper) Membrane holder, which can be attached to a syringe for filtration of various types of specimens, particularly urine. (Lower) Package of membranes; different sizes with various mesh sizes are available, depending on the clinical specimen and suspected organism size.
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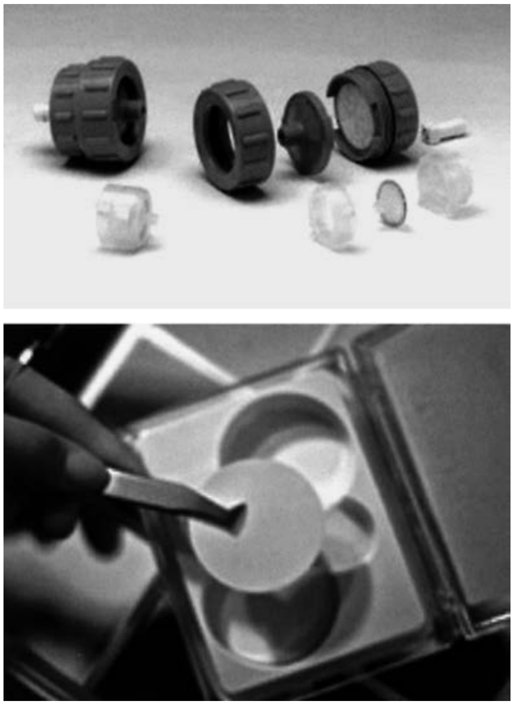

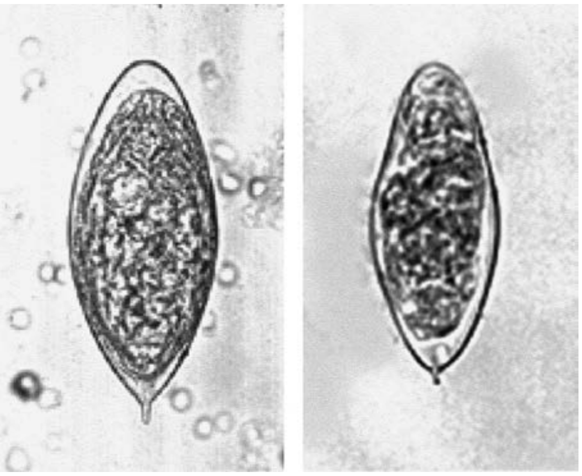

Figure 5.10 *Schistosoma haematobium* eggs from a urine filtration. Note the terminal spines on the eggs. Since the specimens were not preserved prior to filtration, determination of the viability of the eggs is possible. The viability information should be conveyed to the physician as a part of the report.
doi:10.1128/9781555819002.ch5.f10

Schistosomiasis

A membrane filter technique for the recovery of *Schistosoma haematobium* eggs is also useful (32) (Fig. 5.9 and 5.10).

Membrane Filter Technique

1. Collect a urine sample in a container (urine should be well mixed before step 2).
2. With a syringe, draw up 1 ml of urine into the syringe barrel.
3. Fill the rest of the barrel volume with air.
4. Attach the filter holder containing an 8-mm-pore-size Nuclepore membrane filter to the syringe.
5. Express the urine through the filter.
6. Remove the filter, and place it on a microscope slide face down.
7. Moisten the filter with 0.85% NaCl.
8. Examine the filter under ×100 power for the presence of eggs.

The efficiency of the polycarbonate membrane filtration technique for detecting *S. haematobium* eggs in urine is increased by using a pore size of 14 µm and the suction of a water jet pump. Egg concentrations of 1 egg in >1,000 ml of urine can be detected. Viability can be assessed after filtration by staining with trypan blue. This technique is highly recommended in light infections with small numbers of eggs in which previous diagnostic methods have not confirmed the infection (33).

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6

Sputum, Aspirates, and Biopsy Material

Expectorated sputum
Induced sputum
Aspirates
Lungs and liver
Lymph nodes, spleen, liver, bone marrow, spinal fluid, eyes, and nasopharynx
Cutaneous ulcer
Biopsy material
Skin
Lymph nodes
Muscle
Rectum and bladder

Since *Pneumocystis jirovecii* (previously *P. carinii*) has been reclassified with the fungi, this organism is no longer included in this book or discussed in terms of organism, disease, pathogenesis, and diagnosis. However, other organisms such as the microsporidia can be stained using silver stains, so these methods and references have been kept in this chapter and will periodically refer to *P. jirovecii*.

Expectorated Sputum

Although it is not one of the more common specimens, expectorated sputum may be submitted for examination for parasites. Organisms in sputum that may be detected and may cause pneumonia, pneumonitis, or Loeffler's syndrome include the migrating larval stages of *Ascaris lumbricoides*, *Strongyloides stercoralis*, and hookworm; the eggs of *Paragonimus* spp.; *Echinococcus granulosus* hooklets; and *P. jirovecii* (now classified with the fungi), *Entamoeba histolytica*, *Entamoeba gingivalis*, *Trichomonas tenax*, *Cryptosporidium* spp., and possibly the microsporidia (now classified with the fungi) (1). In a *Paragonimus* infection, the sputum may be viscous and tinged with brownish flecks, which are clusters of eggs ("iron filings"), and may be streaked with blood (Fig. 6.1).

Collection and Examination of the Specimen

Sputum is usually examined as a wet mount (saline or iodine), using low and high dry power (×100 and ×400). The specimen is not concentrated before preparation of the wet mount. If the sputum is thick, an equal amount of 3% sodium hydroxide (or undiluted chlorine bleach) can be added; the specimen is thoroughly mixed and then centrifuged. NaOH should not be used if *Entamoeba* spp. or *T. tenax* is being sought; the protozoa will be destroyed. After centrifugation, the supernatant fluid is discarded and the sediment can be examined as a wet mount with saline or iodine. If examination has to be delayed for any reason, the sputum should be fixed in 5 or 10% formalin to preserve helminth eggs or larvae or in one of the fecal fixatives to be stained later for protozoa. Usually, sputum is not a recommended specimen for the diagnosis of *P. jirovecii*; however, if such a specimen is accepted by the laboratory, other stains such as silver methenamine, Giemsa, or immunoassay reagents are used. If *Cryptosporidium* is suspected (rare), acid-fast or immunoassay techniques normally used for stool specimens can be used (see chapters 3 and 15) (2–5). Trichrome stains of material

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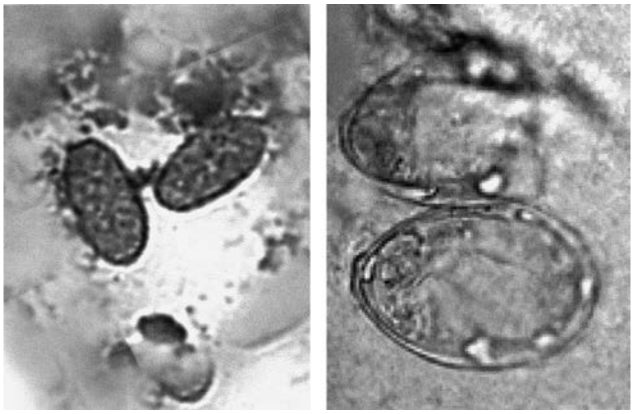


Figure 6.1 *Paragonimus* spp. eggs. (Left) Operculated eggs in a sputum specimen. (Right) Eggs photographed at a higher magnification. doi:10.1128/9781555819002.ch6.f1

may aid in differentiating *E. histolytica* from *E. gingivalis*, and Giemsa stain may better define larvae and juvenile worms.

A sputum specimen should be collected properly so the laboratory receives a "deep sputum" sample for examination rather than a specimen that is primarily saliva from the mouth rather than from the lower respiratory passages. If the sputum is not induced, the patient can be instructed as follows.

1. Expectorated sputum specimens are collected after the patient is instructed in the appropriate measures to ensure high-quality specimens, including prior mouth washing with hydrogen peroxide and exclusion of saliva from specimens. Try to obtain the specimen early in the morning, when the chances of obtaining a deep sputum specimen are increased.
2. Transport the specimen to the laboratory in clean, closed containers as quickly as possible. Select any blood-tinged, viscous areas for sampling.
3. If the specimen is uniformly mucoid:
   - A. *Wear gloves when handling specimens.*
   - B. Remove a 1.0-ml portion to a 15-ml conical tube.
   - C. Add 1.0 ml of mucolytic agent such as Sputalysin (Sputalysin Stat-Pack dithiothreitol solution [Behring Diagnostics, Inc.]) which has been prepared as specified by the manufacturer. This reagent can be stored unopened at room temperature until the expiration date. Date and store working solution at 4°C (include the expiration date). Discard working solution after 48 h. Prepare working solution by removing 1.0 ml from the 10-ml bottle and diluting with 9.0 ml of sterile water.
   - D. Incubate at room temperature for 15 min.
   - E. Add 2.0 ml of phosphate buffer (pH 6.8; M/15 [0.067 M]).
   - F. Centrifuge the material at 500 × *g* for 5 min.
   - G. Decant supernatant fluid, and use sediment to prepare wet mounts and smears.
   - H. Quality control measures should include the following.
     - a. Ensure that the saline and mucolytic agent are free of contamination (particulate matter) as determined by a clear appearance. If cloudy, discard and make new working solution.
     - b. Control the trichrome stain for each new set of reagents with a specimen containing blood to ensure that white cells stain with purple nuclei and blue-green cytoplasm. If cells do not stain appropriately, change reagents.
     - c. With each new lot of Giemsa stain or new buffer, check the stain with a specimen containing blood to ensure that red cells stain grayish, white cell nuclei stain red-purple, and cytoplasm stains bluish. If cells do not stain appropriately, check the stock stain and buffer to find cause.
     - d. Yeast-containing material may be used as a positive control for silver staining.
     - e. Both *P. jirovecii*-positive and *P. jirovecii*-negative and yeast-positive material should be used for immune-specific staining (check with suppliers).
     - f. Stains cannot be evaluated if controls do not stain appropriately. A stain is not within acceptable results when:
       - (i) *P. jirovecii* does not stain or stains black without delineation of "parentheses."
       - (ii) Other fungi and actinomycetes do not stain.
     - g. Store stock stain solutions in area away from light; discard any reagent (prior to the expiration date) that is cloudy or appears contaminated.
     - h. Evaluate the fluorescence microscope for correct light wavelength, using commercially available quality control slides.
     - i. Record all quality control results.

Procedure for Examination of Sputum

1. *Wear gloves when performing these procedures.*

2. For expectorated sputum (no addition of mucolytic agent):
   - A. Using a Pasteur pipette, place 1 or 2 drops (50 µl) on one side of a 2- by 3-in. (1 in. = 2.54 cm) glass slide, and cover with a 22- by 22-mm (no. 1) coverslip.
   - B. Place a second drop on the slide, add 1 drop of saline, and cover with a coverslip.
3. Expectorated sputum (treated with a mucolytic agent) can be resuspended in 100 µl of saline; place 1 drop on a 2- by 3-in. slide, and cover with a coverslip.
4. Save the untreated specimen and remaining treated specimen for permanent smear preparation if stains are required.
5. Examine the entire 22- by 22-mm coverslip wet preparation under low light with the 10× objective to detect eggs, larvae, oocysts, or amebic trophozoites.
6. If results are inconclusive, prepare smears for permanent staining.
   - A. Place 1 drop of sediment in the center of each of three 1- by 3-in. glass slides, add 2 to 3 drops of fecal fixative to one of the slides, and mix/spread the material with the tip of the pipette.
   - B. Allow all three smears to air dry a minimum of 30 min (they can be incubated for drying at 37°C).
   - C. Trichrome stain the slide fixed in fecal fixative.
   - D. Fix the other two air-dried smears in methanol; stain one with Giemsa and the other with a modified acid-fast stain. The modified trichrome stain may have to be used if infection with microsporidia is suspected (6, 7).
   - E. Put immersion oil on stained smears; examine the Giemsa-stained smear with the 10× objective and the trichrome-stained smear with the 50× oil objective if available; otherwise, use the 100× oil objective. Read at least 300 oil immersion fields (total magnification, ×1,000) before reporting the specimen as negative.
   - F. The methenamine silver stain can be used; however, expectorated sputum is generally not recommended as an acceptable specimen for the recovery and identification of *P. jirovecii.*
7. Report any organisms found as follows.
   - A. Give genus and species, if necessary, after confirmation with permanent stain.
   - B. "No parasites found" in expectorated sputum is considered a normal/negative report; therefore, call the physician if any organisms are found.

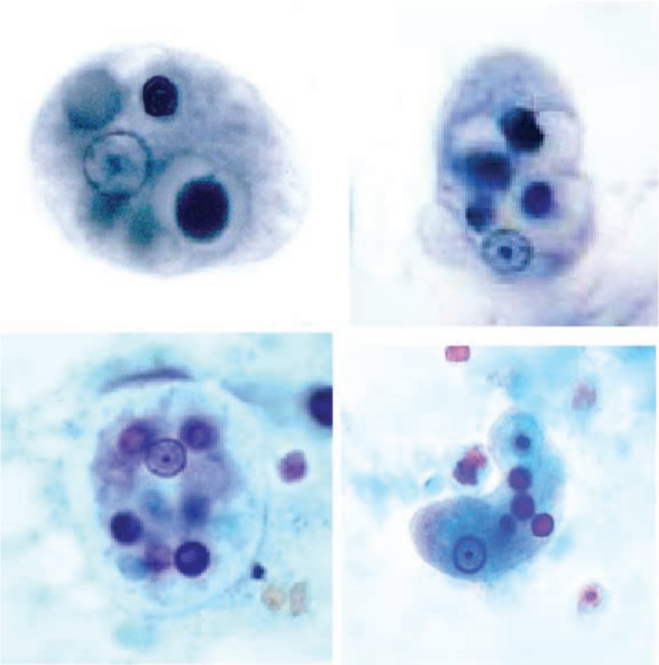


Figure 6.2 (Upper) *Entamoeba gingivalis* containing ingested polymorphonuclear leukocytes (PMNs) within large vacuoles. (Lower) *Entamoeba histolytica* containing ingested red blood cells (RBCs). Note: the nuclear characteristics are very similar; however, the vacuole sizes are quite different (large vacuoles contain ingested PMNs, small vacuoles contain ingested RBCs). doi:10.1128/9781555819002.ch6.f2

Note Care should be taken not to confuse *E. gingivalis*, which may be found in the mouth and saliva, with *E. histolytica*, which could result in an incorrect suspicion of pulmonary abscess. *E. gingivalis* usually contains ingested polymorphonuclear leukocytes, while *E. histolytica* may contain ingested red blood cells but not polymorphonuclear leukocytes (Fig. 6.2). *T. tenax* would also be found in saliva from the mouth and thus would be an incidental finding and normally not an indication of pulmonary problems (Fig. 6.3).

Induced Sputum

Concentrated stained preparations of induced sputum specimens are commonly used to detect *P. jirovecii* and differentiate trophozoite and cyst forms from other possible causes of pneumonia, particularly in AIDS patients (8). Organisms must be differentiated from other fungi such as *Candida* spp. and *Histoplasma capsulatum.* Although induced sputum specimens have been used successfully in the diagnosis of *P. jirovecii* in some institutions, they have not been useful in others. This difference may be due in part to careful adherence to specimen rejection criteria. If the clinical evaluation of a patient suggests *P. jirovecii*

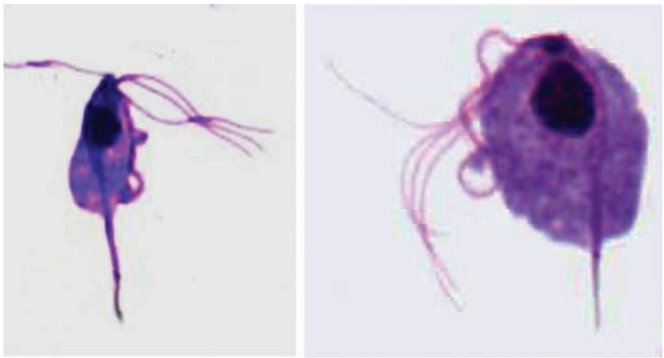


Figure 6.3 (Left) *Trichomonas tenax* from the mouth (stained with Giemsa stain). (Right) *Trichomonas vaginalis* from a genital specimen (stained with Giemsa stain). Note the large nucleus in *T. tenax* and the fact that this flagellate is somewhat smaller than *T. vaginalis.* doi:10.1128/9781555819002.ch6.f3

pneumonia and the induced sputum specimen is negative, a bronchoalveolar lavage fluid specimen should be evaluated with the stains presented in this protocol or newer molecular methods (9).

Collection and Examination of the Specimen

Induced sputum specimens are collected by pulmonary or respiratory therapy staff after patients have used appropriate cleansing procedures to reduce oral contamination. Nebulizing procedures are generally determined by the respiratory therapy staff collecting the specimens. The induction protocol is critical for the success of the procedure, and it is mandatory for well-trained individuals to be involved in the recovery of organisms. Patients with *Pneumocystis* pneumonia usually have dry, nonproductive coughs. Organisms are rarely detected in expectorated sputum, which is not accepted by many laboratories as a clinically relevant specimen. In cooperation with the pulmonary staff, the laboratory processing the specimens must establish a protocol for this diagnostic procedure. Induced sputum specimens are most useful for detection of *P. jirovecii* in HIV-infected individuals, because others have fewer, less readily detected organisms. Stains that can be used for organism detection include the rapid methenamine silver stain, the rapid Giemsa stain (Diff-Quik or Giemsa Plus), the Giemsa stain, and immune-specific staining (10).

Preparation of the Specimen Prior to Staining

1. *Wear gloves when handling specimens.*
2. Specimens which contain mucus should be treated with a mucolytic agent by adding the agent in a 1:1 ratio with the specimen, usually 2 or 3 ml, and incubating the specimen at room temperature for 15 min. Large-volume specimens usually are watery. These specimens (up to 20 to 25 ml) should be concentrated by centrifugation prior to the addition of a mucolytic agent.
3. Centrifuge the specimen at 500 × *g* for 5 min in capped centrifuge tubes and closed carriers.
4. Decant supernatant fluids into a disinfectant solution (1:10 dilution of bleach).
5. If the sediment contains a significant amount of blood, treat a portion (one-half to one-third) with a red-cell lytic agent such as saponin or Lyse in one-half to one-third of the volume of the sediment, leave at room temperature for 5 min, and recentrifuge.
6. Decant supernatant fluid from treated specimens.
7. Use Pasteur pipettes to resuspend remaining sediment.
8. Using 1- by 3-in. glass slides, place drops of sediment in the center of each slide. For specimens treated to lyse red cells, prepare two smears, one from material before lytic treatment and one from the treated specimen.
9. Spread the drops with the pipette so that they are thin and even.
10. Air dry slides, and dip them in methanol prior to Giemsa or silver staining. Fix slides for immune-specific staining as specified in the package insert directions.

Reagents Used in Staining Procedures

1. Mucolytic agent: Sputalysin Stat-Pack dithiothreitol solution (Behring Diagnostics, Inc.). Red cell lytic agent: saponin (Aldrich, Milwaukee, WI; ICN Biochemicals, Costa Mesa, CA), Lyse (Curtin Matheson Scientific, Inc.), or Hematall LA-Hgb reagent (Fisher Scientific).
2. Rapid Giemsa stain: Diff-Quik (Baxter Scientific Products) or Wright's Dip Stat (Medical Chemical). Follow the manufacturer's instructions or use Giemsa stain.
3. Giemsa stain: azure B alcoholic stock (Harleco, Philadelphia, PA) diluted 1:20 with phosphate buffer containing 0.01% Triton X-100.
4. Buffers prepared from stock buffers for the Giemsa stain:
   - A. Alkaline buffer $NaHPO_4$ M/15 solution is 9.5 g of Na_2HPO_4 dissolved in 1 liter of distilled water.
   - B. Acid buffer $NaHPO_4$ M/15 solution is 9.2 g of $N\,Na_2HPO_4 \cdot H_2O$ dissolved in 1 liter of distilled water.

These buffers can be kept for 12 months. To prepare buffered water for the stain, add 39 ml of acid buffer and 61 ml of alkaline buffer to 900 ml of water.

Reagents for Rapid Methenamine Silver Stain (Microwave) (6)

Chromic Acid (10% Solution)

Chromic acid (CrO_3)	100.0 g
Distilled deionized water	1,000.0 ml

Solution is stable for up to 1 year.

Methenamine (3% Solution)

Hexamethylenetetramine, USP [$(CH_2)_6N_4$]	12.0 g
Distilled deionized water	400.0 ml

Solution is stable for up to 6 months.

Silver Nitrate (5% Solution)

Silver nitrate ($AgNO_3$)	5.0 g
Distilled deionized water	100.0 ml

Solution is stable for 1 to 6 months at 4°C.

Sodium Borate (Borax) (5% Solution)

Sodium borate ($Na_2B_4O_7 \cdot 10H_2O$)	5.0 g
Distilled deionized water	100.0 ml

Solution is stable for up to 1 year.

Sodium Bisulfite (1% Solution)

Sodium bisulfite ($NaHSO_3$)	1.0 g
Distilled deionized water	100.0 ml

Solution is stable for up to 1 year.

Gold Chloride (1% Solution)

Gold chloride	5.0 g
Distilled deionized water	500.0 ml

Solution is stable for up to 1 year.

Sodium Thiosulfate (5% Solution)

Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)	50.0 g
Distilled deionized water	1,000.0 ml

Solution is stable for up to 1 year.

Stock Light Green

Light green, S.F. (yellow) (C.I. no. 42095)	0.2 g
Distilled deionized water	100.0 ml
Glacial acetic acid (CH_3COOH)	0.2 ml

Solution is stable for up to 1 year.

Working Light Green

Stock light green	10.0 ml
Distilled deionized water	40.0 ml

Solution is stable for up to 1 month.

Ethanol, 100% (Absolute) and 95%

Methanol, 100% (Absolute)

Xylene or Xylene Substitute

All reagents can be stored at 25°C except for the silver nitrate, which must be refrigerated. Although stable for up to 6 months, silver nitrate may have to be discarded and replaced after storage for 1 month.

Procedure for Rapid Methenamine Silver Stain (Microwave) (11)

1. Place specimen smear slides and control slides on stain rack.
2. Add 10% chromic acid to cover smears, and let stand for 10 min.
3. During this time, prepare working methenamine-silver nitrate by placing the following in order in a plastic Coplin jar: 20 ml of 3% methenamine, 1 ml of 5% silver nitrate, 1.5 ml of 5% sodium borate, and 17 ml of distilled deionized water.
4. Wash slides with distilled deionized water.
5. Cover slides with 1% sodium bisulfite for 1 min.
6. Wash slides with water, place them in the plastic Coplin jar containing methenamine-silver nitrate, and cover with cap.
7. Place in microwave oven at 50% power for approximately 35 s, rotate 90°, and heat for another 35 s; leave slides in hot liquid for 2 min. Solution should turn brown (fresh reagent may appear almost clear if the stock methenamine is freshly prepared; with time and use, the working solution will turn more brown).
8. If a water bath is used, heat it to 80°C. Place plastic Coplin jar containing stain reagents in bath for 6 min prior to placing slides in jar, add slides, and leave jar in bath for an additional 5 min. Solution should turn brown (fresh reagent may appear almost clear if the stock methenamine is freshly prepared; with time and use, the working solution will turn more brown).
9. Remove slides; wash in distilled deionized water.
10. Dip slides up and down in 1% gold chloride, wash in distilled deionized water, and place on rack again.
11. Cover slides with 5% sodium thiosulfate for 1 min.
12. Wash in distilled deionized water.
13. Cover with 0.2% fast green in acetic acid, counterstain for 1 min; use working solution.
14. Wash with distilled deionized water, and stand slides on end to drain and dry.
15. Examine with oil immersion or mount slides.
16. Fill Coplin jar used for staining with bleach (full strength), and let stand for at least 1 h.

Reagents for Rapid Methenamine Silver Stain (No Microwave) (12)

In another rapid methenamine silver-staining procedure, a 5% (rather than 10%) chromic acid solution is used, the gold chloride is present at 0.2% (rather than 1%), the sodium thiosulfate is present at 2% (rather than 5%), and the microwave oven is not used (12). Another difference from the microwave method involves fixation of the smears prior to staining. Heat fix all smears at 70°C for 10 min on a heating block for specimens that could contain *Mycobacterium tuberculosis*. Place the air-dried smears in absolute methyl alcohol for 5 min. Fix a control smear (microsporidia, fungus, or *P. jirovecii*) at the same time. It is mandatory that control smears be used every time patient specimens are stained.

Chromic Acid (5% Solution)

Chromic acid (CrO_3)	5 g
Distilled water	100 ml

Solution is stable for up to 1 year.

Methenamine (3% Solution)

Hexamethylenetetramine, USP [$(CH_2)_6N_4$]	3 g
Distilled water	100 ml

Solution is stable for up to 1 year.

Sodium Bisulfite (1% Solution)

Sodium bisulfite ($NaHSO_3$)	1 g
Distilled water	100 ml

Solution is stable for up to 1 year.

Sodium Thiosulfate (Hypo) (2% Solution)

Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)	2 g
Distilled water	100 ml

Solution is stable for up to 1 year.

Stock Light Green

Light green, S.F. (yellow) (C.I. no. 42095)	0.2 g
Distilled water	100 ml
Glacial acetic acid (CH_3COOH)	0.2 ml

Solution is stable for up to 1 year.

Silver Nitrate (5% Solution)

Silver nitrate ($AgNO_3$)	5 g
Distilled water	100 ml

Solution is stable for 1 to 6 months at 4°C.

Sodium Borate (Borax) (5% Solution)

Borax, photographic or USP ($Na_2B_4O_7 \cdot 10H_2O$)	5 g
Distilled water	100 ml

Solution is stable for up to 1 year.

Gold Chloride (0.2% Solution)

Gold chloride, 1% solution ($AuCl \cdot HCl \cdot 3H_2O$)	10 ml
Distilled water	40 ml

This solution is stable for 1 year; however, when toning begins to fail and the organisms come out too black, it should be changed.

Note The 1% gold chloride solution is made from ampoules and is diluted as specified in the accompanying directions (dilute with tap water).

Stock Methenamine-Silver Nitrate

Silver nitrate, 5% solution	5 ml
Methenamine, 3% solution	100 ml

A white precipitate forms but immediately dissolves on shaking. Clear solutions remain stable for months at refrigerator temperature (4°C).

Working Light Green

Light green, stock solution	10 ml
Distilled water	40 ml

Solution is stable for up to 1 month.

Working Methenamine-Silver Nitrate

Borax, 5% solution	2 ml
Distilled water	25 ml
Mix, and add:	
Methenamine-silver nitrate, stock solution	25 ml

Caution The working methenamine-silver nitrate solution must be prepared fresh each time the stain is run. Do not try to reuse, even for a stain run immediately following one previously performed.

Procedure for Rapid Methenamine Silver Stain (No Microwave) (12)

1. While slides are fixing, fill one Coplin jar (with a lid) with 5% chromic acid. In a second, screw-cap Coplin jar, prepare the working methenamine-silver nitrate solution.
2. Place fixed slides into chromic acid, and put both Coplin jars into the 80°C water bath. Warm the jars and their contents in a stream of hot water for approximately 30 s before placing them in the water bath, to prevent the jars from cracking. Place smears into the heated 5% chromic acid, and incubate them in the water bath for 2 min (oxidation step).
3. Wash slides briefly in running tap water.
4. Place slides into 1% sodium bisulfite for 30 s.
5. Rinse slides in three changes of distilled water.
6. Place slides into the jar of working methenamine-silver nitrate solution in the 80°C water bath for 4.5 min (reduction step).
7. Rinse slides with three changes of distilled water. Wipe backs of slides with a paper towel to remove excess methenamine-silver nitrate.

8. Tone in 0.2% gold chloride for 30 s.
9. Rinse in three changes of distilled water.
10. Place slides into 2% sodium thiosulfate for 30 s (removes reduced silver).
11. Rinse in three changes of distilled water.
12. Counterstain in working light green solution for 30 s.
13. Rinse in three changes of distilled water.
14. Dehydrate and clear for 30-s intervals in two changes each of 95% ethyl alcohol, 100% ethyl alcohol, and xylene or xylene substitute, respectively.
15. Mount slides in Permount.

The fungi generally stain gray to black; *P. jirovecii* exhibits a delicately stained wall, usually brownish or grayish, which is somewhat transparent. Structures described as "parentheses" are usually seen and are stained dark gray or black (Fig. 6.4). If the organisms are too dark, it is probably time to change the solutions. It is also important to prepare the smears so that the material is thin and evenly spread on the glass slide. Glycerin, red blood cells, and mucin stain rose taupe to dark gray. The inner parts of mycelia and hyphae stain "old rose." The background usually appears pale green. Microsporidial spores stain dark gray to black; the horizontal or diagonal "stripe" or polar filament is not visible in every spore (Fig. 6.4). Various background colors are visible, depending on the stain used, thickness of the specimen, and tissue source of the specimen.

Note The examination of specimens for the diagnosis of microsporidia is not often considered to be a STAT procedure and may be handled in the anatomic pathology division. The stained smears can be difficult to read and interpret, hence the need for available experienced personnel. However, special modified trichrome stains for the microsporidia are often performed in the microbiology laboratory; the procedure is not difficult, but examination of the stained smears is very labor intensive.

Rapid Giemsa Stain (Diff-Quik or Giemsa Plus)

1. Keep stain solutions in dropper bottles. Place 1 or 2 drops of red stain (solution 1) on specimen smear and control slide (normal blood film), hold for 10 s, and drain.
2. Add drops of blue (solution 2), hold for 10 s, drain, and rinse very briefly with deionized water.
3. Stand slides on end to drain and air dry.
4. Slides must be examined with oil or mounted with mounting medium.

Giemsa Stain

Make Giemsa working solution fresh each day. Discard and make new solution after 10 slides have been stained in a Coplin jar. Additional directions can be found in chapter 7.

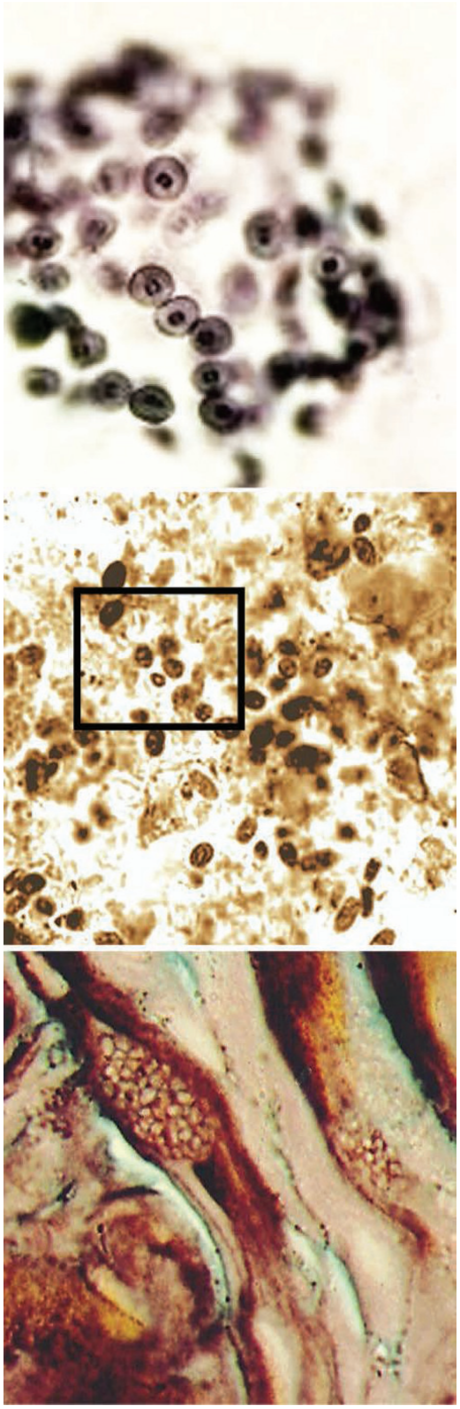


Figure 6.4 (Upper) *Pneumocystis jirovecii*. Note that the "parentheses" are visible within the cell wall (methenamine silver stain). (Middle) Microsporidial spores in a stool specimen stained with silver stain (methenamine silver stain). Note the spore with a polar tubule (horizontal line through the spore) within the black box; there are other examples of spores containing the polar tubule throughout the image. (Bottom) Microsporidian spores within an ocular biopsy specimen (methenamine silver stain). In this image the spores are clustered together and polar tubules are not visible. doi:10.1128/9781555819002.ch6.f4

1. Place 2 ml of Giemsa (azure B) stain in a Coplin jar. Remove stain from stock stain in bottle with a clean, dry pipette.
2. Add 40 ml of phosphate buffer (pH 7.0 to 7.2) containing 0.01% Triton X-100.
3. Place fixed specimen smears and control smears in Giemsa stain for 30 min.
4. Remove slides, dip in phosphate buffer, stand slides on end, and allow to drain and air dry.
5. Examine with oil or mount slides.

Giemsa Staining for Microsporidia

1. Internal morphology may be difficult to interpret (spores with polar tubules).
2. Giemsa stain may be more relevant for specimens other than stool (urine, sputum, eye).
3. Perform modified trichrome (Ryan blue or Weber green) stains on stool.

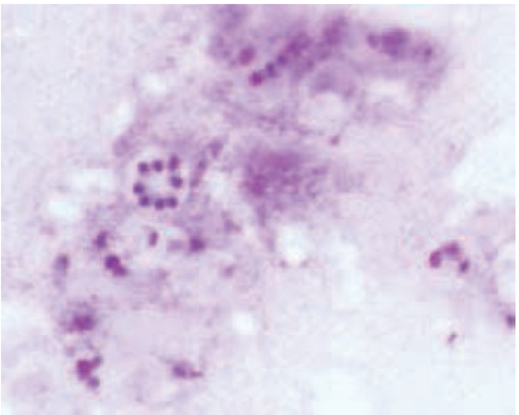
Potential Problems with Stain Interpretation

In the procedures in which the spores are stained, spores and fungi may appear very much alike. If rare organisms are seen, it may be almost impossible to differentiate microsporidia from various fungi, particularly if no budding organisms are seen. Although the microsporidia are now classified with the fungi, they will continue to be covered in both sections of this book.

Although a second type of stain can be used (Giemsa, which stains the spores), the organisms may be very difficult to differentiate from cellular debris. Consequently, few laboratories rely on the Giemsa stain alone, especially when staining specimens for *Pneumocystis* or the microsporidia (Fig. 6.5).

Other methods such as fluorescent-antibody and immunoperoxidase techniques have been used for *P. jirovecii* (13–17). The development of tissue culture procedures for *P. jirovecii* may lead to additional diagnostic procedures for this infection. Immunoassay reagents are also available (14–18) and are being widely used in many diagnostic laboratories.

Figure 6.5 *Pneumocystis jirovecii* trophozoites within the cyst wall (stained with Giemsa stain). Note that the cyst wall is not visible when Giemsa stain is used. Other trophozoites can also be seen in the background. doi:10.1128/9781555819002.ch6.f5



When noninduced sputum and the direct fluorescent-antibody technique were used, the sensitivity was 55%; this is within the range reported in the literature for the diagnosis of *Pneumocystis* pneumonia from induced sputum (19). Although detection of *P. jirovecii* has increased with the use of PCR, particularly in sputum specimens, some workers recommend that sputum samples of HIV-infected patients be tested by both PCR and immunofluorescence. The results of PCR in this patient group could be misleading without careful clinical evaluation (20). However, the level of sensitivity seen using PCR and other molecular tests indicates that these procedures should be considered for patients in whom *Pneumocystis* pneumonia is suggestive clinically and the specimen is negative by the immunofluorescence test (21, 22).

Quality Control Measures

Quality control measures for all stains should include the following, and control slides must be incorporated into all stain procedures.

1. Examine a control slide for each stain prior to examination of specimen stains. The stain intensity of controls will be a guide to the stain appearance of organisms in specimens.
2. Examine stained specimen smears by systematically moving from field to field until the majority of the smear has been covered (total area for silver stain).
3. In Giemsa stains, *Pneumocystis* trophozoite clumps of various sizes may be detected. In large clumps, it may be difficult to differentiate individual organisms. *Look at organisms at the edges of clumps, and look for small, more dispersed clumps.*
4. In silver- or other cyst wall-stained smears, look for the various cyst forms, including those that show dark centers, cup-shaped cysts, and cysts with foldlike lines (they look like "punched-in" ping-pong balls). If dark-staining organisms appear more oval, look carefully for budding forms, which indicate that the organisms are yeasts. *It is important to review thinner areas of the smear for microsporidial spores.*
   - **A.** *P. jirovecii* cysts: 70% should have delicately stained walls, usually brown or gray. They appear somewhat transparent, with structures described as "parentheses" staining black; these curved structures are usually thick (much

thicker than the cyst wall) rather than thin like a line drawing.

  B. Other fungi and actinomycetes: gray to black. Microsporidial spores stain dark gray to black; some of the spores may contain the polar tubule (horizontal or diagonal stripe), but they are more difficult to see in the silver stain than in the modified trichrome stains.

  C. Glycogen, mucin, and red blood cells: rose taupe to gray.

  D. Background: pale green (from the light green counterstain).

5. Detection of *P. jirovecii* in specimen smears from one type of stain always suggests a careful examination of the other stain, hopefully to confirm the identification.

6. Retain all positive stained specimen slides and control slides for reference.

Reporting Smear Results

1. Report *P. jirovecii* as follows (high dry power; total magnification, ×400).
  A. "No *Pneumocystis jirovecii* seen."
  B. "*Pneumocystis jirovecii* seen" (no quantitation should be included).

2. Report other fungi that may be present as follows (low power; total magnification, ×100).
  A. "No mycotic elements seen."
  B. "Budding yeasts" or "Budding yeasts and pseudohyphae resembling *Candida* species" are quantitated as few, moderate, or many.
  C. Hyphae are reported with no quantitation:
    a. "Septate hyphae seen."
    b. "Nonseptate hyphae seen."

3. Report actinomycetes (oil immersion; total magnification, ×1,000) as follows.
  A. "No filamentous branching bacteria seen."
  B. "Filamentous branching bacteria seen" (no quantitation).

4. Report microsporidia (oil immersion; total magnification, ×1,000) as follows.
  A. "No microsporidial spores seen."
  B. "Microsporidial spores seen; identification to genus not possible." However, if the specimen is stool or other gastrointestinal tract specimens, the organisms are probably *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis*; a note containing this information can be attached to the report.

Notes on Staining Procedures

1. Use at least two stains for detection and identification of *P. jirovecii*. With traditional histochemical stains, a trophozoite stain such as Giemsa and a cyst wall stain such as methenamine-silver nitrate are recommended.

2. There are many cyst wall stains in addition to the one described, and there are other modifications of the silver stain (12, 23).

3. Stain effectiveness varies. Other counterstains may be used; a counterstain with Giemsa is useful if referral slides will be submitted for examination.

4. When selecting a cyst wall stain, consider stain quality, reagent stability, and potential testing frequency (including STAT requests). Toluidine blue O stains vary in dye lots and in the stability of sulfation reagents (24). In the modified procedure, the sulfation reagent made of glacial acetic acid and concentrated sulfuric acid presents disposal problems.

5. The rapid silver stain described is a modification of the procedure described by Brinn (11). Heating the chromic acid may cause nonspecific staining, making the background too dark. Also, buildup of silver on the stain container may interfere with staining. Bleach should be added to the stain jar after staining, and jars should be scrubbed periodically with a brush. Additional tips for getting good stain results include the following.
  A. Before use, inspect glassware to ensure that it does not have residual silver deposits.
  B. Use fresh reagents for each run (5% chromic acid, 1% sodium bisulfite, methenamine-silver nitrate working solution, and 2% sodium thiosulfate).
  C. Distilled deionized water must be used throughout the procedure, including slide rinses (microwave procedure).
  D. Methenamine-silver nitrate working solution must be clear; if it becomes opaque at any time, the reduction step may take longer or may not occur at all. Check the distilled deionized water source, and prepare fresh methenamine-silver nitrate working solution.

6. If mounted slides appear opaque or cloudy, the dehydration and clearing with xylene (or xylene substitute) were not adequate. Soak slides in xylene to remove the coverslips. Using fresh ethanol and xylene, repeat the dehydration steps.

7. Degenerating polymorphonuclear leukocytes may resemble *P. jirovecii*.

8. Monoclonal antibodies specific for human strain *P. jirovecii* are now available. The commercial systems vary; some are indirect stains, and some are direct stains. Reports with all systems have been variable. Many laboratories are now using these immunospecific stains.

9. Select an organism stain and a cyst wall stain or immunospecific stain; use of a pair of stains will help avoid both false-negative and false-positive reporting.

10. In addition to organism detection, cytocentrifuge preparations of sputum can be used to determine cell populations for further patient evaluation.

Aspirates

The examination of aspirated material for the diagnosis of parasitic infections may be extremely valuable, particularly when routine testing methods have failed to demonstrate the organisms. These types of specimen should be transported to the laboratory immediately after collection. Aspirates include liquid specimens collected from a variety of sites where organisms might be found. Aspirates most commonly processed in the parasitology laboratory include fine-needle aspirates and duodenal aspirates. Fluid specimens collected by bronchoscopy include bronchoalveolar lavage fluid and bronchial washings.

Procedural details for sigmoidoscopic aspirates and scrapings for the recovery of *E. histolytica* are presented in chapter 5. Techniques for preparation of duodenal aspirate material are also presented in that chapter.

Specimens Obtained from Aspiration Procedures

Fine-needle aspirates are often collected by cytopathology staff, who process the specimens, or they may be collected and sent to the laboratory directly for slide preparation and/or culture. Suggested stains are Giemsa and methenamine silver for *P. jirovecii*, Giemsa for *Toxoplasma gondii* (25), trichrome for amebae, modified acid-fast stains for *Cryptosporidium* (2), and modified trichrome stains for the microsporidia (see chapter 3).

Aspirates of cysts and abscesses to be evaluated for amebae may require concentration by centrifugation, digestion, microscopic examination for motile organisms in direct preparations, and cultures and microscopic evaluation of stained preparations.

Duodenal aspirates to be evaluated for *S. stercoralis*, *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*). *Cryptosporidium* spp., *Cyclospora cayetanensis*, or the microsporidia require concentration by centrifugation prior to microscopic examination for motile organisms and permanent stains (26–29).

Cyst aspirates to be evaluated for hydatid "sand" (daughter cysts, small protoscolices, hooklets) can be examined as wet direct mounts after centrifugation. Several stains can be used to visualize the hooklets; one of the best is the Ryan blue modified trichrome stain for the microsporidia (see chapter 3). The stained smears can be examined using routine microscopy with transmitted light (30).

Bone marrow aspirates to be evaluated for *Leishmania* amastigotes, *Trypanosoma cruzi* amastigotes, or *Plasmodium* spp. require staining with Giemsa or another blood stain (31, 32).

Fluid specimens collected by bronchoscopy may be lavages or washings, with bronchoalveolar lavages preferred. Specimens usually are concentrated by centrifugation prior to microscopic examination of stained preparations. Organisms included here which may be detected are *P. jirovecii*, *Toxoplasma gondii*, and *Cryptosporidium* spp.

Lungs and Liver

Pneumocystosis

Although formerly classified with the sporozoa, *P. jirovecii* has been reclassified with the fungi. It is an important cause of pulmonary infections, particularly in patients who are immunosuppressed as a result of therapy or from congenital or acquired immunologic deficiencies (33, 34). Clinically, the infection may involve both lungs diffusely, may be localized, or may be disseminated. For immunosuppressed patients, in whom the disease may progress very quickly, correct specimen collection and rapid diagnostic techniques are very important. The organisms can be demonstrated in stained impression smears of lung material obtained by open or brush biopsy. *P. jirovecii* can also be seen in stained smears of tracheobronchial aspirates, although examination of lung tissue is more likely to reveal the organisms. Sputum specimens are generally considered unacceptable for the recovery of *P. jirovecii*; however, in patients with severe, progressive disease, such specimens may be acceptable. To avoid the possibility of false-negative results, acceptance of sputum specimens should be carefully monitored and reviewed. Also, even for patients with fulminant disease, multiple specimens may have to be submitted to recover and identify the organisms. With a number of the stains that are available, the cyst walls stain but the cyst contents do not (Fig. 6.4); however, only the methenamine silver stain is discussed here (15).

Amebiasis

Examination of aspirates from lung or liver abscesses may reveal trophozoites of *E. histolytica* (Fig. 6.6); demonstration of the organisms is often very difficult. In many cases, serologic confirmation is recommended (35). Liver aspirate material should be taken from the margin of the abscess rather than the necrotic center (Fig. 6.7). The organisms are often trapped in the viscous pus or debris and may not exhibit typical motility. The Amoebiasis Research Unit, Durban, South Africa, has recommended using proteolytic enzymes to free the organisms from the aspirate material.

The digestion technique is performed as follows.

1. A minimum of two separate portions of exudate should be removed (more than two are recommended). The first portion of the aspirate, usually

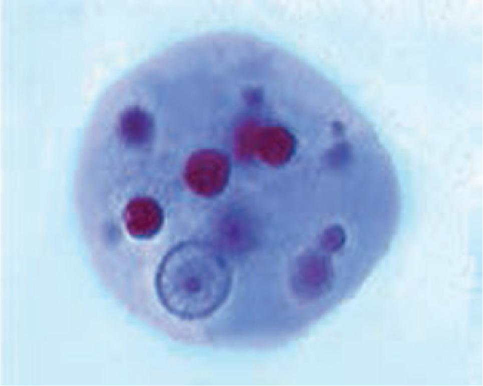
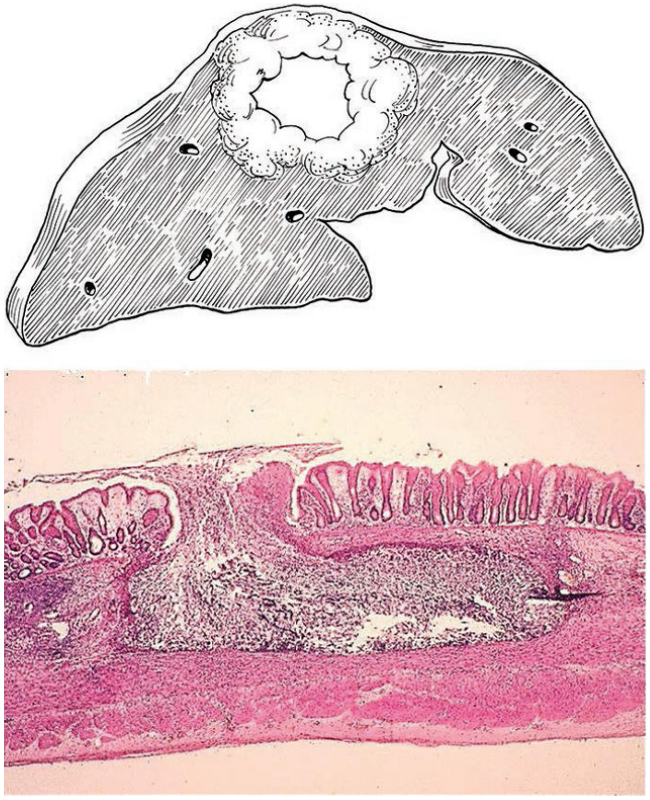


Figure 6.6 *Entamoeba histolytica* containing ingested red blood cells within the cytoplasm; morphologically this organism is *E. histolytica*, and this would be the species designation if organisms were isolated from liver abscess material (trichrome stain). doi:10.1128/9781555819002.ch6.f6

Figure 6.7 (Upper) Liver abscess caused by *Entamoeba histolytica*. Amebae would be found at the advancing margin of the lesion; the last portion of the aspirated material might reveal the organisms. (Illustration by Sharon Belkin.) (Lower) Amebic abscess; note the "flask"-shaped ulcer. doi:10.1128/9781555819002.ch6.f7



yellowish white, rarely contains organisms. The last portion of the aspirated abscess material is reddish and is more likely to contain amebae. The best material to examine is that obtained from the actual wall of the abscess.

2. Add 10 U of streptodornase per ml of thick pus; incubate this mixture for 30 min at 37°C, and shake repeatedly.
3. Centrifuge the mixture at 500 × *g* for 5 min. The sediment may be examined microscopically as wet mounts or used to inoculate culture media. Some of the aspirate can be mixed directly with a fecal fixative on a slide, allowed to air dry, stained, and examined as a permanent stained smear.

Hydatid Disease

Aspiration of cyst material for the diagnosis of hydatid disease is a dangerous procedure and is normally performed only when open surgical techniques are used for cyst removal. Aspirated fluid usually contains hydatid sand (intact and degenerating protoscolices, hooklets, and calcareous corpuscles) (Fig. 6.8). Some older cysts contain material that resembles curded cottage cheese, and the hooklets may be very difficult to see. Some of this material can be diluted with saline or 10% KOH; usually, protoscolices or daughter cysts will have disintegrated. However, the diagnosis can be made by seeing the hooklets, which can be stained using the Ryan blue modified trichrome stain (see chapter 3) (Fig. 6.9).

Examination of hydatid cyst material is carried out as follows.

1. If the cyst material is fluid, centrifuge at 500 × *g* for 3 min.
2. Carefully remove some of the sediment and prepare a wet mount.
3. Examine the material under low (100×) and high dry (430×) power (remember to use low light intensity, since some of the material may be very transparent).
4. If the cyst material is more viscous or solid, the material can be mixed with saline or 10% KOH and then centrifuged at 500× *g* for 3 min.
5. Some of the very viscous material can be placed on a glass slide (undiluted). Another glass slide can be placed on top of the first (material will now lie between the two slides). Rub the glass slides back and forth over each other and listen for a grating sound (like grains of sand being scratched). If this occurs, you may be hearing evidence of the presence of calcified hooklets. Place the material from the smears (may be diluted with saline) under the microscope to try to confirm the presence of hooklets

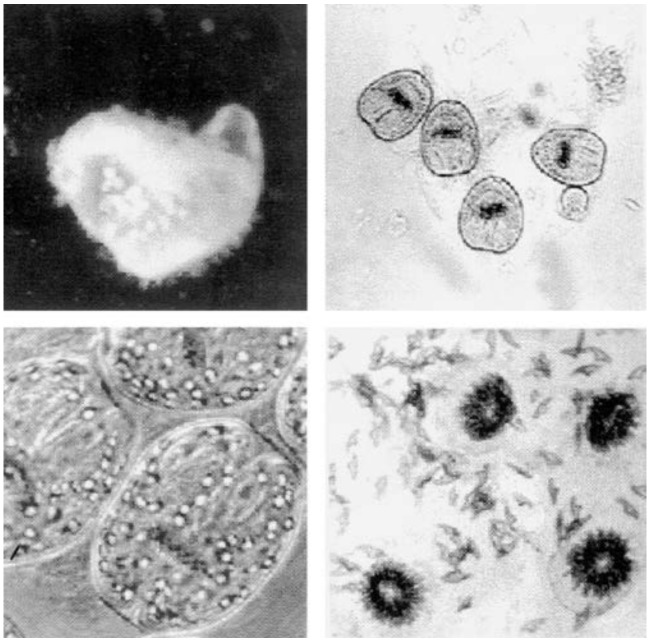


Figure 6.8 Hydatid disease (*Echinococcus granulosus*). (Upper left) Hydatid cyst with protoscolices budding off from the germinal layer. (Upper right) Immature protoscolices, with the dark area being the hooklets. (Lower left) Higher magnification of the protoscolices taken from the hydatid cyst fluid. (Lower right) Hooklets from disintegrating protoscolices. Reprinted from reference 3. doi:10.1128/9781555819002.ch6.f8

(which may be extremely difficult to see with only low power).

6. A dried smear of the cyst aspirate can be stained using the Ryan blue modified trichrome stain (see chapter 3). The hooklets will stain reddish-purple in color; remember they are quite small, measuring 20 to 40 µm long.

Note Remember, the absence of protoscolices or hooklets does not rule out the possibility of hydatid disease, since some cysts are sterile and contain no protoscolices and/or daughter cysts. Review of the cyst wall from pathology (tissue sections) should be able to confirm the diagnosis.

Lymph Nodes, Spleen, Liver, Bone Marrow, Spinal Fluid, Eyes, and Nasopharynx

African Trypanosomiasis, Leishmaniasis, Chagas' Disease, Primary Amebic Meningoencephalitis, Granulomatous Amebic Encephalitis, Amebic Keratitis, and Microsporidial Keratitis

Material from lymph nodes, spleen, liver, bone marrow, spinal fluid, eye specimens, or nasopharynx may be examined for the presence of parasites and should be processed as follows.

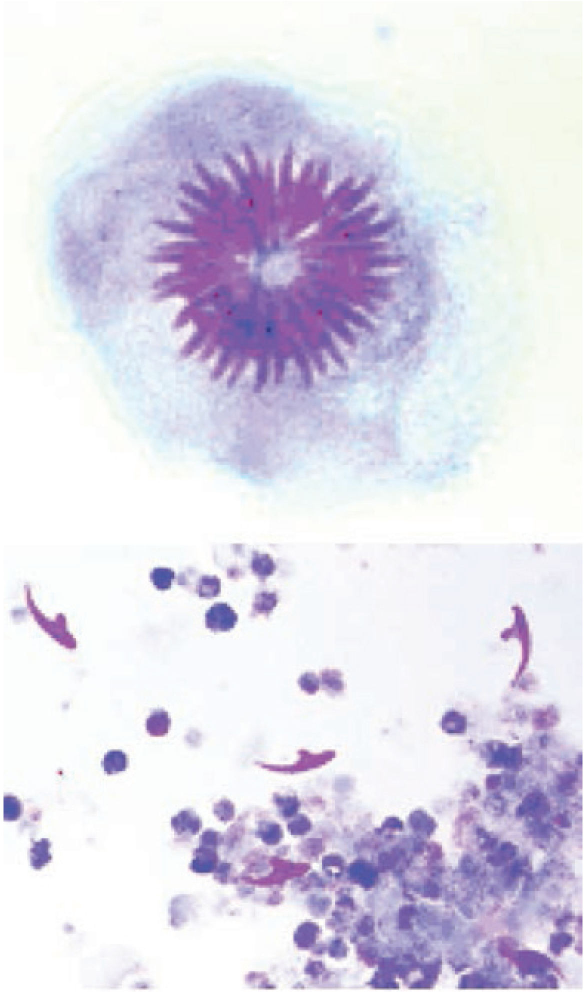


Figure 6.9 Hydatid disease (*Echinococcus granulosus*). (Upper) Hydatid protoscolex revealed by Ryan modified trichrome stain. (Lower) Hydatid hooklets revealed by Ryan blue modified trichrome stain (reprinted from reference 30). doi:10.1128/9781555819002.ch6.f9

1. A portion of the fluid material can be examined under low (×100) and high dry (×400) power as a wet mount (diluted with saline) for the presence of motile organisms. ***Spinal fluid should not be diluted before examination.***
2. Impression smears from tissues should be prepared and stained with Giemsa or another blood stain. The material is pressed between two slides, and a smear is formed when the slides are pulled apart (one across the other).
3. The smears are allowed to air dry and then processed like a thin blood film (fixed in absolute methanol and stained with Giemsa stain or one of the other blood stains).
4. Appropriate culture media should be inoculated with any remaining material (see chapter 8).

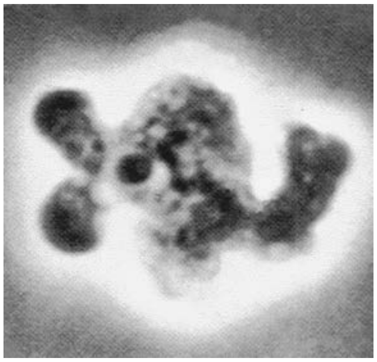


Figure 6.10 *Naegleria fowleri.* Note the large karyosome and lobate pseudopodia. Spinal fluid should be examined on a slide, not in a counting chamber, where protozoan trophozoites could mimic white blood cells. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch6.f10

5. If microsporidia are suspected, modified trichrome stains (Ryan blue, Weber green) can be used; calcofluor white and immunoassay methods (currently under development) are also excellent options (36–43).

Primary amebic meningoencephalitis is rare, but the examination of spinal fluid may reveal the amebae, usually *Naegleria fowleri* (Fig. 6.10). Granulomatous amebic encephalitis is caused by *Acanthamoeba* spp., *Balamuthia mandrillaris*, or *Sappinia diploidea*. Uncentrifuged sedimented spinal fluid should be placed under a coverslip on a slide and observed for motile amebae; smears can also be stained with Wright's, Giemsa, or one of the other blood stains (including the rapid stains). Spinal fluid, exudate, or tissue fragments can be examined by light microscopy or phase-contrast microscopy. Care must be taken not to confuse leukocytes with actual organisms and vice versa. The appearance of the spinal fluid may vary from cloudy to purulent (with or without red blood cells), with a cell count from a few hundred to over 20,000 white blood cells (primarily neutrophils) per ml. ***Failure to find bacteria in this type of spinal fluid should alert one to the possibility of primary amebic meningoencephalitis.*** These organisms can be isolated from tissues or soil with special media (see chapter 8). **Note** When spinal fluid is placed in a counting chamber, any organisms that settle to the bottom of the chamber will tend to round up and look very much like white blood cells. For this reason, it is better to examine the spinal fluid on a slide directly under a coverslip, not in a counting chamber.

A rapid method for the diagnosis of *Acanthamoeba* or microsporidial keratitis involves the use of calcofluor white, which is a chemofluorescent dye with an affinity for the polysaccharide polymers of amebic cysts and microsporidial spores (Fig. 6.11 and 6.12). The following method has proven to be very successful for examination of corneal scrapings or biopsy material (42).

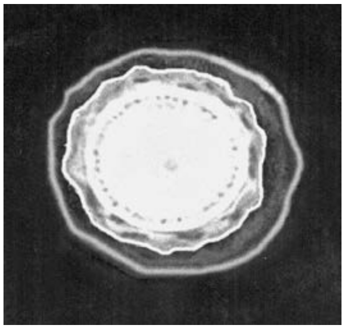
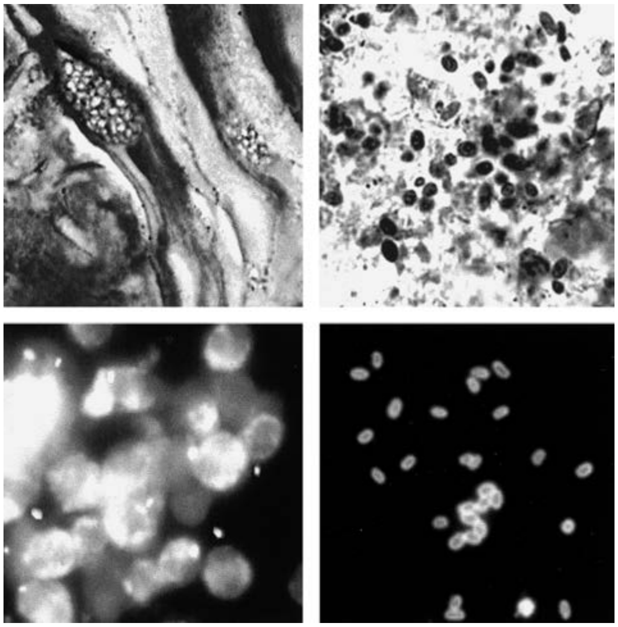


Figure 6.11 *Acanthamoeba* cyst. Note the fluorescence of the cyst wall. Trophozoites do not fluoresce when calcofluor white is used. doi:10.1128/9781555819002.ch6.f11

1. Place corneal scrapings on slides, and air dry them.
2. Fix the slides in absolute methyl alcohol for 3 to 5 min.
3. Add several drops of solution (0.1% calcofluor white and 0.1% Evans blue dissolved in distilled water); leave on for 5 min.
4. Turn the slide on its side, and allow excess stain to run off onto paper towels.

Figure 6.12 Microsporidial spores. (Upper left) Spores in a corneal scraping specimen; (upper right) spores in a fecal specimen (both images stained with silver stain). (Lower left) Microsporidial spores in a urine sediment; (lower right) spores from nasopharyngeal aspirate (both images stained using calcofluor white). doi:10.1128/9781555819002.ch6.f12



5. Add a coverslip, and examine under the fluorescence microscope for pale blue chemofluorescence of the amebic cysts (will not stain trophozoite). The microsporidial spores usually stain brighter; however, fluorescence can vary between 1+ and 3+.

Note Select UV irradiation with an exciter filter which transmits the 365-nm group of intense mercury spectral emission lines (Zeiss UGI or G365). View through a barrier filter which removes UV while emitting visible blue light and longer wavelengths (Zeiss no. 41 or LP420).

Cutaneous Ulcer

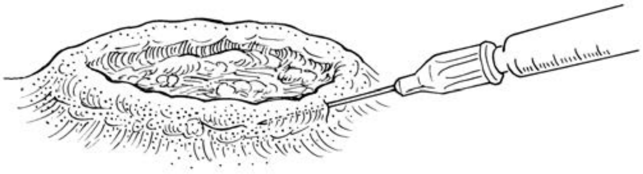
Leishmaniasis

Material containing intracellular *Leishmania* organisms must be aspirated from below the ulcer bed through the uninvolved skin, not from the surface of the ulcer (Fig. 6.13). It is very important that the surface of the ulcer be thoroughly cleaned before specimens are taken; any contamination of the material with bacteria or fungi may prevent recovery of organisms from culture. Cutaneous ulcers can be seen in Fig. 6.14.

Biopsy Material

Biopsy specimens are recommended for the diagnosis of tissue parasites. The following procedures may be used for this purpose in addition to standard histologic preparations: impression smears and teased and squash preparations of biopsy tissue from the skin, muscle, corneas, intestines, liver, lungs, and brain. Tissue to be examined by permanent sections or electron microscopy should be fixed as specified by the laboratories that will process the tissue. In certain cases, examination of a biopsy specimen may be the only means of confirming a suspected parasitic infection. Specimens that are going to be examined as fresh material rather than as tissue sections should be kept moist in saline and submitted to the laboratory immediately.

Figure 6.13 Proper way to aspirate material from below the ulcer bed (*Leishmania* spp.); sterile saline (0.5 to 1.0 ml) can be injected under the ulcer prior to aspiration. (Illustration by Sharon Belkin.)
doi:10.1128/9781555819002.ch6.f13



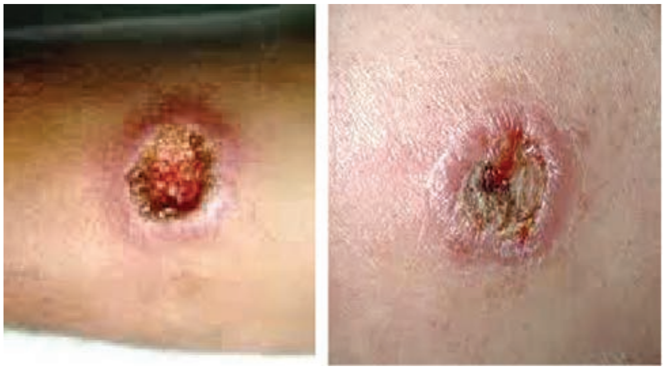


Figure 6.14 *Leishmania* cutaneous lesions.
doi:10.1128/9781555819002.ch6.f14

Importance of Biopsy Specimens

Success in detection of parasites in tissue depends in part on specimen collection and on the presence of sufficient material to perform the recommended diagnostic procedures. Biopsy specimens are usually quite small and may not be representative of the diseased tissue. Examination of multiple tissue samples often improves diagnostic results. To optimize the yield from any tissue specimen, examine all areas and use as many procedures as possible. Tissues are obtained by invasive procedures, many of which are very expensive and lengthy; consequently, these specimens deserve the most comprehensive procedures possible. ***It is also important to remember that if the tissue specimen is not sufficient for all diagnostic procedures requested, the procedures should be prioritized after consultation with the physician.***

Submission of Specimens

Tissue submitted on a sterile sponge dampened with saline in a sterile container may be used for cultures of protozoa after mounts for direct examination or impression smears for staining have been prepared. If cultures for parasites will be included, sterile slides should be used for smear and mount preparation.

1. Use sterile slides for impression smears; slides can be either autoclaved or prepared by an alternative method in which they are soaked in 95% ethyl alcohol and flamed prior to use.
2. Use sterile (autoclaved or flamed) forceps for handling tissue.
3. Place tissue in sterile petri dish to examine macroscopically and select a sample for microscopic evaluation. Provided that it is kept sterile, minced tissue can be used.
   A. If biopsy tissue is several millimeters to a centimeter in size, select the specimen from an abnormal area (granulomatous lung or ulcerated

area of intestinal tissue). Generally, the tissue fragments are too small to permit such a judgment to be made.

   B. If several small pieces of tissue that look alike are submitted, use one piece. However, if they look different, use one of each type for microscopic examination. Again, sometimes this assessment to make.

4. Prepare impression smears.

   A. Blot the tissue sample on sterile toweling. If the sample size is sufficient, cut the tissue with a scalpel (very sharp cut) and use the cut surface to touch the slide.

   B. Press tissue against the sterile slide, lift, and press again. Turn the sample over, and press the other end against the slide to make two more impressions. ***Keep impressions close together to speed the screening process with the microscope.***

   C. Air dry and fix smears in absolute methanol for 1 min for subsequent blood stain, methenamine-silver nitrate, modified acid-fast, and modified trichrome staining. If the amount of tissue is sufficient, multiple smears should be prepared for each stain (Fig. 6.15).

   D. Place wet slide in Schaudinn's fixative for subsequent trichrome staining.

   E. Fix the slide as specified by the manufacturer for immunospecific staining.

5. Teased preparations are made as follows.

   A. Place sample in the bottom of a plastic petri dish, and cover with 2 to 4 drops of saline.

   B. Gently tease tissue apart with needles, or hold the tissue with forceps while pulling apart with a scalpel or needles.

   C. Put a cover on dish, and leave at room temperature for 30 min.

6. Squash preparations are made as follows.

   A. Using a scalpel, cut selected tissue portions into very fine pieces.

   B. Place a piece of tissue on a 1- by 3-in. slide, add 1 drop of saline, cover with a second 1- by 3-in. slide, and hold together with membrane clips (from a surgical supply company). If these are not available, paper clips can be used but are not as efficient. ***You can also press the slides together with your fingers, but always wear gloves and be careful not to contaminate the microscope stage with tissue fluids.***

7. Skin scrapings should be submitted in a small vial.

8. Prepare cultures to demonstrate the following organisms (see chapter 8): *E. histolytica*, *Acanthamoeba* and *Naegleria* spp., and *Leishmania* spp.

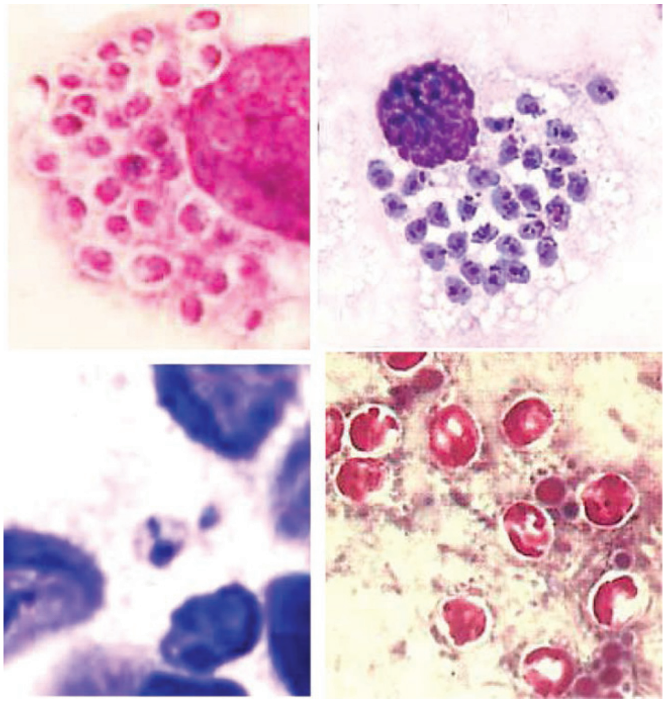


Figure 6.15 (Upper) *Leishmania donovani* amastigotes in a cell; the one on the right is stained with Giemsa stain. (Lower left) *Leishmania* amastigote; note the nucleus and bar (primitive flagella). (Lower right) *Cryptosporidium* spp. oocysts stained with modified acid-fast stain. Note that the sporozoites are visible within some of the oocysts.
doi:10.1128/9781555819002.ch6.f15

9. Mouse passage for *Toxoplasma gondii*:

   A. Grind tissue in 0.85% NaCl into a fine suspension.

   B. Inject 0.2 to 0.4 ml of suspension intraperitoneally into three to five mice of any laboratory strain weighing ~20 g.

   C. Maintain mice in isolation.

   D. Every day, check for signs of central nervous system dysfunction; if symptoms are detected, perform an autopsy on the animal.

Examination of Specimens

1. Examination of impression smears is summarized in Table 6.1.

2. Examine skin snips in teased preparations for detection of microfilariae of *Onchocerca volvulus* and *Mansonella streptocerca* as follows.

   A. Tease the small bit of tissue apart in a few drops of saline to release the microfilariae.

   B. Remove drops of saline to a 1- by 3-in. glass slide, cover with a no. 1 coverslip, and examine under low light for microfilariae.

   C. For a permanent preparation, place 100% methanol under the coverslip to fix filariae,

TABLE 6.1 Examination of impression smears

Tissue	Possible parasite	Stain
Lung	*Pneumocystis jirovecii* (now classified with the fungi)	Giemsa, methenamine silver or other cyst wall stain, optical brightening agent (calcofluor), immunospecific reagent
	Microsporidia	Modified trichrome, acid-fast stain, Giemsa, optical brightening agent (calcofluor), methenamine silver, EM[a]
	Toxoplasma gondii	Giemsa, immunospecific reagent
	Cryptosporidium spp.	Modified acid-fast stain, immunospecific reagent
	Entamoeba histolytica	Giemsa, trichrome
Liver	*Toxoplasma gondii*	Giemsa
	Leishmania donovani	Giemsa
	Cryptosporidium spp.	Modified acid-fast stain, immunospecific reagent
	Pneumocystis jirovecii (now classified with the fungi)	Giemsa, methenamine silver or other cyst wall stain, optical brightening agent (calcofluor), immunospecific reagent
	Entamoeba histolytica	Giemsa, trichrome
Brain	*Naegleria* sp.	Giemsa, trichrome
	Acanthamoeba sp.	Giemsa, trichrome
	Balamuthia mandrillaris	Giemsa, trichrome
	Sappinia diploidea	Giemsa, trichrome
	Entamoeba histolytica	Giemsa, trichrome
	Toxoplasma gondii	Giemsa, immunospecific reagent
	Microsporidia (*Encephalitozoon* spp.)	Modified trichrome, acid-fast stain, Giemsa, optical brightening agent (calcofluor), methenamine silver, EM
Skin	*Leishmania* spp.	Giemsa
	Onchocerca volvulus	Giemsa
	Mansonella streptocerca	Giemsa
	Acanthamoeba sp.	Giemsa, trichrome
	Entamoeba histolytica	Giemsa, trichrome
Nasopharynx, sinus cavities	Microsporidia	Modified trichrome, acid-fast stain, Giemsa, optical brightening agent (calcofluor), methenamine silver, EM
	Acanthamoeba sp.	Giemsa, trichrome
	Naegleria sp.	Giemsa, trichrome
Intestine		
Small intestine	*Cryptosporidium* spp. (both small and large intestine)	Modified acid-fast stain, immunospecific reagent
	Cyclospora cayetanensis	Modified acid-fast stain
Jejunum	Microsporidia (*Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*)	Modified trichrome, acid-fast stain, Giemsa, optical brightening agent (calcofluor), methenamine silver, EM
Duodenum	*Giardia lamblia* (*G. duodenalis*, *G. intestinalis*)	Giemsa, trichrome
Colon	*Entamoeba histolytica*	Giemsa, trichrome
Cornea, conjunctiva	Various genera of microsporidia	Acid-fast stain, Giemsa, modified trichrome, methenamine silver, optical brightening agent (calcofluor), EM
	Acanthamoeba sp.	Giemsa, trichrome, calcofluor for cysts
Muscle	*Trichinella* spp.	Wet examination, squash preparation
	Microsporidia (*Pleistophora* sp., *Trachipleistophora* sp., *Brachiola* sp.)	Modified trichrome, acid-fast stain, Giemsa, optical brightening agent (calcofluor), methenamine silver, EM

[a] EM, electron microscopy. Although EM tends to be very specific, this method is not particularly sensitive, since the sample sampled and examined tends to be so small.

partially dry, remove the coverslip, and stain with Giemsa or one of the other blood stains (including the rapid stains).

3. Examine squash preparations for detection of *Trichinella* spp. in muscle microscopically at low power (×100) and under low light.
4. Examine scrapings of skin for *Sarcoptes scabiei* (scabies) microscopically at low power (×100) and under low light. Confirmation at high dry power (×400) may be necessary (Fig. 6.16). See chapter 35 on arthropods for specific/detailed skin-scraping protocol for scabies.
5. Inoculate cultures with ground tissue suspensions (to release organisms from the cells).
   - A. Place a small tissue sample in a sterile tissue grinder (Ten Broeck or Dounce) in 0.5 ml of sterile saline, and grind until tissue is broken up but not totally liquefied.
   - B. Add several drops of ground tissue to culture medium as follows.
     - a. NNN medium (see chapter 8): add drops of tissue to the bottom of the slant, where they will "pool" with condensed moisture. Incubate at room temperature (isolation of *Leishmania* spp.).
     - b. TYSGM-9 medium (see chapter 8): add drops of tissue to the liquid medium, add 3 drops of the starch suspension, and incubate at a 45 to 50° angle at 35°C for 48 h (isolation of *E. histolytica*).
     - c. Nonnutrient agar plate seeded with bacteria (see chapter 8): add drops to center of seeded agar plate, and incubate at 35 to 37°C (isolation of *Acanthamoeba* or *Naegleria* spp.).
6. Examine cultures (*wear gloves at all times*).
   - A. NNN agar for promastigotes of *Leishmania* spp. (see chapter 8): using a Pasteur pipette, remove a drop of fluid from interface of agar slant and culture tube, place on glass slide, cover with coverslip, and examine microscopically (×400) under low light for motile promastigotes (Fig. 6.17).
   - B. TYSGM-9 medium for amebae (see chapter 8): using a sterile Pasteur pipette, remove 1 or 2 drops of material from interphase of agar and overlay, place on glass slide, cover with coverslip, and examine microscopically (×100) under low light for trophozoite motility.
   - C. Nonnutrient agar plate with lawn of *Escherichia coli* or *Enterobacter* sp. for detection of free-living amebae (see chapter 8): examine microscopically at low power (×100) for changes in bacterial lawn, particularly patches and tracks, indicating that the protozoan trophozoites have ingested bacteria as they move over the agar.

Figure 6.16 *Sarcoptes scabiei* mites from skin scrapings. (Upper) Note the two mites to the left of center. (Lower) Note the two eggs, the small nymph, and the adult mite (photographed at a higher magnification). If the light is too strong when examining the scrapings in saline, the mites will probably not be seen. doi:10.1128/9781555819002.ch6.f16

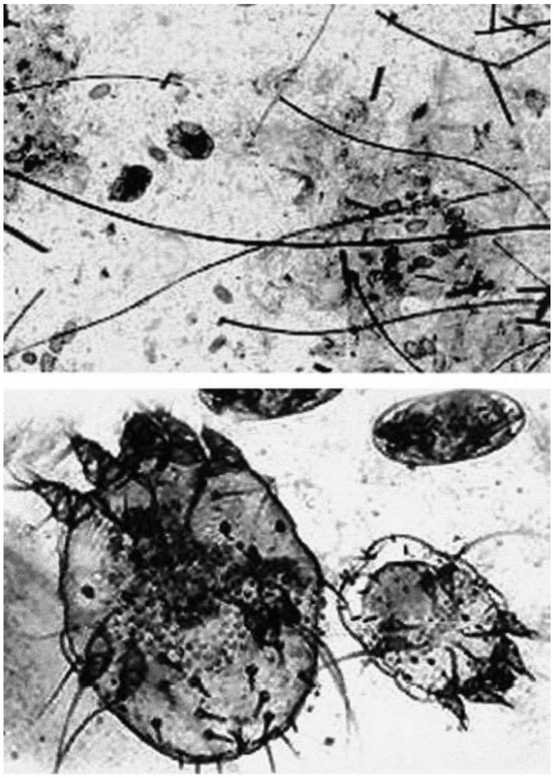
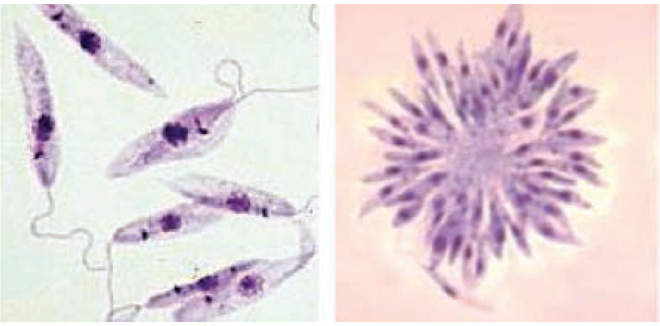


Figure 6.17 *Leishmania* culture sediment. Note the promastigotes from the culture sediment. The typical "rosette" formation in the right image is frequently seen. doi:10.1128/9781555819002.ch6.f17



D. Mouse passage for detection of *Toxoplasma gondii*:
   a. Wear canvas gloves to handle mice; sacrifice the animal(s).
   b. Pin mouse to board, spray with 70% ethyl alcohol, and open peritoneal cavity with sterile scissors.
   c. Using a sterile Pasteur pipette, remove fluid from the peritoneal cavity, and place in small tube or prepare smears.
   d. Using one of the blood stains, prepare stained smears of peritoneal exudate, and examine microscopically at a magnification of ×1,000 for *T. gondii* tachyzoites.
   e. Place mice and all contaminated disposable materials in bag to be autoclaved and destroyed. Place nondisposable materials (scissors) in bag to be autoclaved prior to washing.

7. Correlate all examination results (wet mount, stains, and cultures) to determine presence of organisms.

Results

The majority of the protozoa are found on the permanent stained smears (impression smears, touch or squash preparations, or teased preparations). When culture is used, permanent stained smears of the culture medium or sediment may also reveal some of the protozoa. Although infrequently used, material from animals (at autopsy) can be examined as both wet and permanent stained preparations for confirmation of protozoa. Filarial infections may be confirmed by the recovery and identification of microfilariae in skin scrapings and/or biopsy specimens.

If identifications are certain, report the organisms detected; if a presumptive identification is made, confirmation by another laboratory is suggested.

Skin

Onchocerca volvulus and *Mansonella streptocerca*

The use of skin snips is the method of choice for the diagnosis of human filarial infections with *O. volvulus* and *M. streptocerca*. Microfilariae of both species occur chiefly in the skin, although *O. volvulus* microfilariae are on rare occasions found in the blood and occasionally in the urine. For best results, the skin snip specimens should be thick enough to include the outer part of the dermal papillae. Snips may be taken in various ways. A small slice may be cut (using a razor blade) from a skin fold held between thumb and forefinger, or a slice may be taken from a small “cone” of skin pulled up by a needle. The skin snip should be so thin that significant bleeding does not occur, just a slight oozing of fluid. Corneal-scleral punches (either Holth or Walser type) have been found to be successful in taking skin snips of uniform size and depth and an average weight of 0.8 mg (range, 0.4 to 1.2 mg); this procedure is easy to perform and is painless. It has been demonstrated that in African onchocerciasis, it is preferable to take skin snips from the buttock region (above the iliac crest); in Central American onchocerciasis, the preferred skin snip sites are from the shoulders (over the scapula).

Skin snips are placed immediately in a drop of normal saline or distilled water and covered so that they will not dry; teasing the specimen with dissecting needles is not necessary but may facilitate release of the microfilariae. Microfilariae tend to emerge more rapidly in saline; however, in either fluid, the microfilariae usually emerge within 30 min to 1 h and can be examined under low-intensity light with the 10× objective of the microscope. To see definitive morphologic details of the microfilariae, allow the snip preparation to dry, fix it in absolute methyl alcohol, and stain it with Giemsa or one of the other blood stains (Fig. 6.18). For the differential diagnosis of *O. volvulus* and *M. streptocerca*, see chapter 23.

Cutaneous Amebiasis and Cutaneous Leishmaniasis

Skin biopsy specimens for the diagnosis of cutaneous amebiasis and cutaneous leishmaniasis should be processed for tissue sectioning and subsequently stained by the hematoxylin-eosin technique.

Itch Mites (*Sarcoptes scabiei*)

Several genera infect the skin of mammals, with *S. scabiei* being found in humans. *S. scabiei* is microscopic and lives in cutaneous burrows, where the fertilized female deposits eggs (Fig. 6.16). Scabies is transmitted by close contact with infested individuals, including touching, shaking hands, sexual contact, or day care centers with children or the elderly. The usual skin sites that are susceptible to infection are the interdigital spaces, backs of the hands, elbows, axillae, groin, breasts, umbilicus, penis, shoulder blades, small of the back, and buttocks. The most dramatic symptom is intense itching. Scratching commonly causes weeping, bleeding, and sometimes secondary infection. Specific and detailed diagnostic methods for the recovery of mites, eggs, or scybala (fecal pellets) can be found in the chapter on arthropods (chapter 35).

Lymph Nodes

Trypanosomiasis, Leishmaniasis, Chagas’ Disease, and Toxoplasmosis

Material obtained from lymph nodes should be processed for tissue sectioning and as impression smears that should

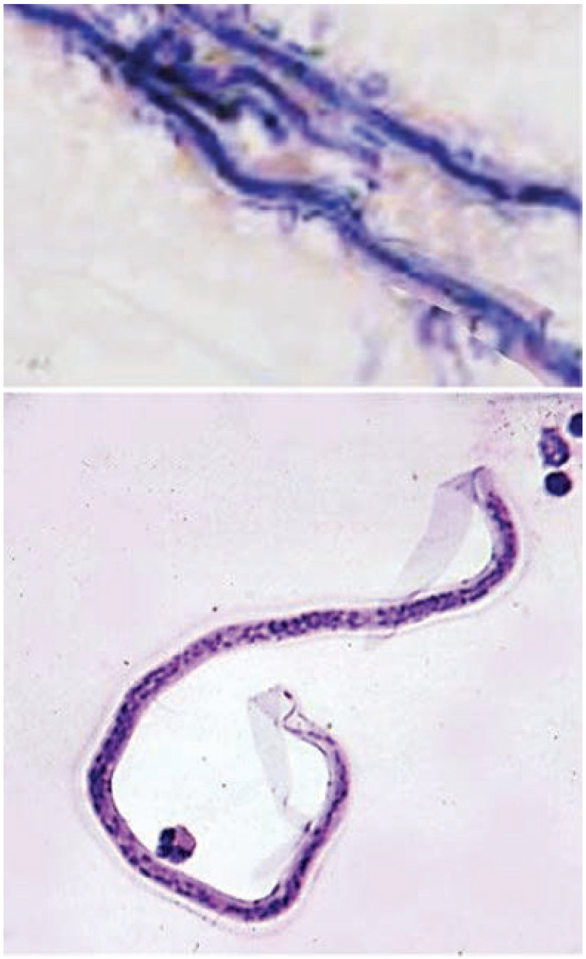


Figure 6.18 Microfilariae (Giemsa stain). (Upper) In this image, the microfilariae from a skin snip saline preparation are visible after staining. (Lower) Microfilariae from blood; note that the characteristic nuclei are more easily seen in this preparation. doi:10.1128/9781555819002.ch6.f18

be processed as thin blood films and stained with Giemsa or other blood stains. Appropriate culture media can also be inoculated, again making sure that the specimen has been collected under sterile conditions.

Muscle

Trichinosis

The presumptive diagnosis of trichinosis is often based on a combination of facts: history of ingestion of raw or rare pork, walrus meat, horse meat, or bear meat; diarrhea followed by edema and muscle pain; and the presence of eosinophilia. Usually, by the time the patient is symptomatic, the suspected food is no longer available for examination. The diagnosis may be confirmed by finding larval *Trichinella* spp. in a muscle biopsy specimen. The encapsulated larvae (which may be few) are easily seen in fresh muscle if small pieces are pressed between two slides and examined under the microscope (Fig. 6.19). Larvae are usually most abundant in the diaphragm, masseter muscle, or tongue and may be recovered from these muscles at necropsy. Routine histologic sections can also be prepared.

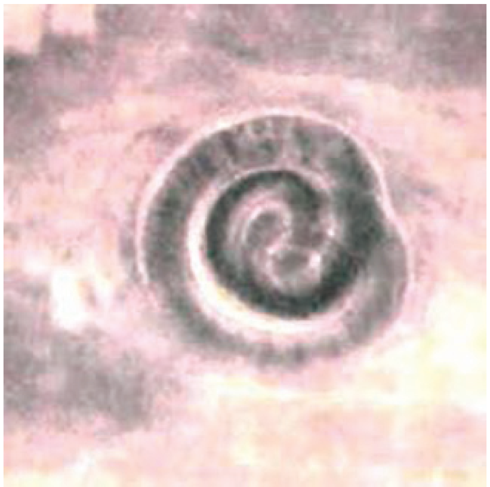
A portion of the specimen can be digested in artificial digestive fluid at 37°C, with the larvae recovered by centrifugation; however, very young larvae might also be digested.

Artificial Digestive Fluid

Pepsin	5 g
Hydrochloric acid (concentrated)	7 ml
Distilled water	1,000 ml

1. Prepare the tissue by grinding (with a tissue grinder or commercial meat grinder or blender). The tissue should be medium grind; do not overgrind, particularly in a blender.
2. Add the tissue to the digestive fluid in the ratio of 1 part tissue to 20 parts fluid in an Erlenmeyer flask.
3. Place the flask in an incubator at 37°C, on a shaker table or magnetic stirrer if available; leave for 12 to 24 h.
4. Add warm water (37°C) to the digestive fluid mixture. (Add enough water to triple the original volume.)
5. Pour the diluted mixture into a Baermann funnel, and add water up to the screen.
6. Allow the mixture to stand for 1 to 2 h; the larvae will settle out in the lower part of the funnel.

Figure 6.19 *Trichinella* spp. larvae in a squash preparation of muscle biopsy tissue; note the encysted larvae. doi:10.1128/9781555819002.ch6.f19



7. Remove a few drops of fluid, and examine the material under the microscope (10× objective) for the presence of larvae.
8. If no larvae are present, centrifuge 50 ml and examine the sediment.

Cestode Larval Stages

Human infection with any of the larval cestodes may present diagnostic problems, and frequently the larvae are referred for identification after surgical removal. In addition to *Echinococcus granulosus* (hydatid disease) and the larval stage of *Taenia solium* (cysticercosis), other larval cestodes occasionally cause human disease. The larval stage of tapeworms of the genus *Multiceps*, a parasite of dogs and wild canids, is called a coenurus and may cause human coenurosis. The coenurus resembles a cysticercus but is larger and has multiple scolices developing from the germinal membrane surrounding the fluid-filled bladder. These larvae occur in extraintestinal locations, including the eye, central nervous system, and muscle (see chapter 25).

Human sparganosis is caused by the larval stages of tapeworms of the genus *Spirometra*, which are parasites of various canine and feline hosts; these tapeworms are closely related to the genus *Diphyllobothrium*. Sparganum larvae are elongated, ribbonlike larvae without a bladder and with a slightly expanded anterior end lacking suckers. They are usually found in superficial tissues or nodules, although they may cause ocular sparganosis, a more serious disease (see chapter 25).

The diagnosis of larval cestodes is frequently facilitated by the recognition of prominent calcareous corpuscles occurring in the tapeworm tissue; specific identification usually depends on referral to specialists.

Rectum and Bladder

Schistosomiasis

Often when a patient has an old, chronic infection or a light infection with *Schistosoma mansoni* or *S. japonicum*, the eggs may not be found in the stool and an examination of the rectal mucosa may reveal the presence of eggs. The fresh tissue should be compressed between two microscope slides and examined under the low power of the microscope (low-intensity light). Critical examination of these eggs should be made to determine whether living miracidia are still found within the egg. Treatment may depend on the viability of the eggs; for this reason, the condition of the eggs should be reported to the physician.

Mucosa from the bladder wall may reveal eggs of *Schistosoma haematobium* when they are not being recovered in the urine. As with rectal biopsy specimens, the eggs in the bladder wall should be checked for viability (fresh, unfixed tissue) (Fig. 6.20).

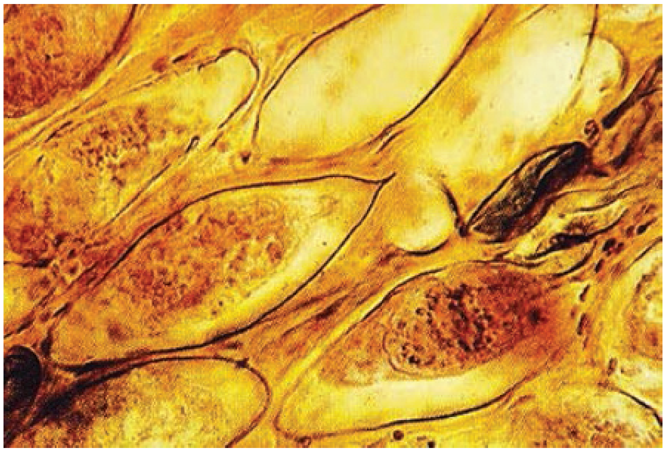


Figure 6.20 *Schistosoma haematobium* eggs seen in a squash preparation of a bladder biopsy specimen. Note the pointed terminal spine. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch6.f20

Viability Testing of Schistosome Eggs

1. With careful observation of the egg, using the 40× objective (low-intensity light), the cilia of the flame cells of the miracidium within the shell can be seen to move in a rapid flickering motion.
2. The eggs within the tissue can be removed (carefully tease the tissue apart in saline solution) and subjected to a hatching procedure. If the miracidia are released from the egg and swim to the top of the hatching flask, this movement is also proof of viability. (The procedure can be found in chapter 4.)

Digestion Procedure in Diagnosis of Schistosomiasis

Small pieces of tissue may be digested in 4% potassium hydroxide (4% sodium hydroxide may be used) for 2 to 3 h at 60 to 80°C. The material may then be concentrated by sedimentation or centrifugation and examined under the microscope for eggs (low power with low-intensity light).

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7

Procedures for Detecting Blood Parasites

Preparation of thick and thin blood films

Thick blood films
Thin blood films
Combination thick and thin blood films (on the same slide)
Combination thick and thin blood films (can be stained as either)
Buffy coat blood films

Staining blood films

Giemsa stain
Wright's stain
General notes on staining procedures

Proper examination of thin and thick blood films

Thin blood films
Thick blood films
Determination of parasitemia

Diagnosis of malaria: review of alternatives to conventional microscopy

QBC microhematocrit centrifugation method
ParaSight F test
NOW malaria test
Flow anti-pLDH *Plasmodium* monoclonal antibodies
Molecular testing
Automated blood cell analyzers

Diagnosis of leishmaniasis: review of alternatives to conventional microscopy

ICT for detection of anti-rK-39 antibodies

Concentration procedures

Cytocentrifugation technique
Knott concentration procedure
Membrane filtration technique
Gradient centrifugation technique
Triple-centrifugation method for trypanosomes

Special stain for microfilarial sheath

Delafield's hematoxylin

Depending on the life cycle, a number of parasites may be recovered in a blood specimen, either whole blood, buffy coat preparations, or various types of concentrations. These parasites include *Plasmodium*, *Babesia*, and *Trypanosoma* species, *Leishmania donovani*, and microfilariae. Although some organisms are motile in fresh, whole blood, species identification is normally accomplished from the examination of both thick and thin permanent stained blood films. Blood films can be prepared from fresh, whole blood collected with no anticoagulants, from anticoagulated blood, or from sediment from the various concentration procedures. Although for many years Giemsa stain has been the stain of choice, the parasites can also be seen on blood films stained with Wright's stain, a Wright/Giemsa combination stain, or one of the more rapid stains such as Diff-Quik (American Scientific Products, McGaw Park, IL), Wright's Dip Stat stain (Medical Chemical Corp., Torrance, CA), or Field's stain. Delafield's hematoxylin stain is often used to stain the microfilarial sheath; in some cases, Giemsa stain does not provide sufficient stain quality to allow differentiation of the microfilariae. A complete discussion of the proper way to examine a blood film is presented later in this chapter. This information is important and particularly relevant when one is examining proficiency testing blood films in the absence of clinical information about patient history and possible etiologic agents.

It is important to remember that standard precautions should be used at all times when blood or body fluids are handled (1). Remember that all requests for malaria diagnosis are considered STAT requests, and specimens should be ordered, collected, processed, examined, and reported accordingly.

Preparation of Thick and Thin Blood Films

Some parasites (microfilariae and trypanosomes) can be detected in fresh blood by their characteristic shape and motility, but specific identification of the organisms requires a permanent stain. Two types of blood films are recommended. Thick films allow a larger amount of blood to be examined, which increases the possibility of detecting light infections (2). However, species identification by thick film, particularly for malaria parasites, can usually be made only by experienced workers. The morphologic characteristics of blood parasites are best seen in thin films, in which the red blood cell (RBC) morphology is preserved and the size relationship between infected and uninfected red cells can be determined

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after staining. This characteristic is often valuable in determining the species of *Plasmodium* present from the thin blood film.

The accurate examination of thick and thin blood films and identification of parasites depend on the use of absolutely clean, grease-free slides for preparation of all blood films. Old (unscratched) slides should be cleaned first with detergent and then 70% ethyl alcohol; new slides should also be cleaned with alcohol before use. When a new box of slides is opened, the slides are coated with a substance that allows them to be pulled apart; these slides should be cleaned before use for preparation of blood films. Do not use cotton; gauze is recommended with 70% alcohol. The advantages and disadvantages of the thin and thick blood films can be seen in Table 7.1.

Blood should be collected immediately on admission or when the patient is first seen in the emergency room and/or clinic; if the initial blood films are negative, collect daily specimens for 2 or 3 additional days (ideally between paroxysms if present; however, there is often no periodicity seen). After a finger stick, the blood should flow freely; blood that has to be "milked" from the finger will be diluted with tissue fluids, decreasing the number of parasites per field. If the specimen is sent directly to the laboratory, thus eliminating laboratory-patient contact, the following approach can be used. Unless you are positive that you will receive well-prepared slides, request a tube of fresh blood (EDTA anticoagulant is preferred/lavender top) and prepare the smears. In general, the use of finger stick blood has declined, particularly in areas of the world where automated hematology instruments have become much more widely used. For detection of stippling, the smears should be prepared within 1 h after the specimen is drawn. After that time, stippling may not be visible on stained films; however, the overall organism morphology will still be acceptable. Potential problems with anticoagulants can be seen in Table 7.2. Although blood films can be prepared from the small amount of blood left in the needle after the venipuncture collection using anticoagulant, it is not recommended for several reasons. The blood tends to clot fairly quickly in the needle, and there are safety recommendations that limit the handling of needles. Therefore, the finger stick and EDTA venipuncture are recommended for collection of specimens for blood film preparation.

The time when the specimen was drawn should be clearly indicated on the tube of blood and also on the result report. The physician will then be able to correlate the results with any fever pattern or other symptoms that the patient may have. However, with travelers who are immunologically naive (have never come in contact with malaria before), there may not be any fever periodicity at all, and the symptoms may be very general and nonspecific for a malaria infection. There should also be some indication on the report that is sent back to the physician that one negative specimen does not rule out the possibility of a parasitic infection.

Note Although most laboratories use commercially available blood collection tubes, the following approach can be used when necessary. EDTA (Sequestrene) can be prepared and tubed as follows. Dissolve 5 g of EDTA in 100 ml of distilled water. Aliquot 0.4 ml into tubes, and evaporate the water. This amount of anticoagulant is sufficient for 10 ml of blood. One can also use 20 mg of EDTA (dry) per tube (20 mg/10 ml of blood). The tube should be filled with blood to provide the proper blood/anticoagulant ratio.

Thick Blood Films

Fresh Blood

To prepare the thick film, place 2 or 3 small drops of fresh blood (no anticoagulant) on an alcohol-cleaned slide. With

TABLE 7.1 Advantages of the thin and thick blood films[a]

Thin blood film advantages	Thick blood film advantages
RBC morphology (size, shape, stippling) can be seen after fixation with methanol prior to staining (Giemsa) or as a part of the staining process (Wright). RBCs are laked in the thick film.	Larger number of parasites per field compared with the thin blood film. RBCs are laked, and so WBCs, platelets, and parasites are visible after staining.
Identification to *Plasmodium* species easier, since the parasite can be seen within the RBC. The size of the parasites within the RBCs can provide information necessary for identification to species.	Phagocytized malaria pigment may be seen within the WBCs, even with a low parasitemia.
Parasitemia can be calculated from the thin film; determination of parasitemia is mandatory for all species of *Plasmodium* and is particularly important for monitoring therapy for *P. falciparum*. Parasitemia should be reported with every set of positive blood films for malaria. It is also recommended that parasitemia be calculated for *Babesia* sp. infections.	Stippling may be seen in a well-stained thick film. However, this depends on how long the blood has been in contact with anticoagulant if it is not collected as a fresh specimen (finger stick).

[a] NOTE: Although for many years Giemsa stain has been the stain of choice, the parasites can also be seen on blood films stained with Wright's stain, a Wright/Giemsa combination stain, or one of the more rapid stains such as Diff-Quik (American Scientific Products, McGaw Park, IL), Wright's Dip Stat stain (Medical Chemical Corp., Torrance, CA), or Field's stain.

TABLE 7.2 Potential problems of the use of EDTA anticoagulant for the preparation of thin and thick blood films

Potential problem	Comments
Adhesion to the slide; blood falls off slide during staining	Incorrect ratio of anticoagulant to blood; fill tube completely with blood (7 ml or pediatric draw tube).
Distortion of parasites; same type of distortion can also be seen after blood is refrigerated (not recommended)	Prolonged storage of blood in EDTA may lead to distortion: trophozoites (*P. vivax*) and gametocytes (*P. falciparum*) tend to round up, thus mimicking *P. malariae*.
Change in ring form size	Ring forms of *P. falciparum* continue to grow and enlarge, thus resembling rings of the other species. Typical "small" rings appear larger than usual.
Use of EDTA anticoagulant (primarily used by hematology laboratories because the cellular components and morphology of the blood cells are preserved) 1. Blood smears for differentials from acceptable specimens should be prepared within 2 h of collection 2. Blood counts from acceptable venipuncture specimens should be performed within 6 h of collection Underfilling the EDTA blood collection tube can lead to erroneously low blood cell counts and hematocrits, morphologic changes to RBCs, and staining alteration; excess EDTA can shrink red cells; conversely, overfilling the blood collection tube will not allow the tube to be properly mixed and may lead to platelet clumping and clotting.	EDTA prevents coagulation of blood by chelating calcium. Calcium is necessary in the coagulation cascade, and its removal inhibits and stops a series of events, both intrinsic and extrinsic, which cause clotting. In some individuals, EDTA may cause inaccurate platelet results. These anomalies, platelet clumping and platelet satellitism, may be the result of changes in the membrane structure occurring when the calcium ion is removed by the chelating agent, allowing the binding of preformed antibodies. Proper mixing of the whole-blood specimen ensures that EDTA is dispersed throughout the sample. Evacuated blood collection tubes with EDTA should be mixed by 8 to 10 end-to-end inversions immediately following venipuncture collection. Microcollection tubes with EDTA should be mixed by 10 complete end-to-end inversions immediately following collection. They should then be inverted an additional 20 times prior to analysis.
Loss of Schüffner's dots (stippling) in *P. vivax* and *P. ovale*	Schüffner's dots (true stippling) occur in both *P. vivax* and *P. ovale*; in the absence of stippling, identification to the species level may be more difficult or impossible.
Prolonged storage of EDTA blood (room temperature with stopper removed)	The pH, CO_2, and temperature changes may reflect conditions within the mosquito. Thus, exflagellation of the male gametocyte may occur while still in the tube of blood prior to thin and thick blood film preparation. Microgametes may be confused with *Borrelia* or may be ignored as debris. The life cycle can continue as it would within the mosquito.
Release of merozoites from the schizonts into the blood; normally, merozoites are not found outside of the RBCs, in contrast to *Babesia* spp., where rings may be seen outside of the RBCs.	Small rings may be seen outside of the RBCs or appear to be appliqué forms, thus suggesting *P. falciparum*. It is important to differentiate these true rings (both cytoplasmic and nuclear colors) from platelets (uniform color).
Incorrect submission of blood in heparin	EDTA has less impact on parasite morphology than does heparin.

the corner of another slide and using a circular motion, mix the drops and spread them over an area ~2 cm in diameter. Continue stirring for 30 s to prevent the formation of fibrin strands that may obscure the parasites after staining.

Anticoagulant

If blood containing an anticoagulant is used, 2 or 3 drops may be spread over an area about 2 cm in diameter; it is not necessary to continue stirring for 30 s, since fibrin strands do not form. If the blood is too thick or any grease remains on the slide, the blood may flake off during staining. It is far better to make the preparation too thin, rather than too thick.

Allow the thick film to air dry (room temperature) in a dust-free area. Never apply heat to a thick film, since heat will fix the blood, causing the RBCs to remain intact during staining; the result is stain retention and inability to identify the parasites. After the thick films are thoroughly dry, they can be laked (lysed) to remove the hemoglobin. Rupture of the RBCs during laking removes the RBCs from the final stained blood film; the only structures remaining on the thick film are the white blood cells (WBCs), the platelets, and any parasites present. To lake the films, place them in buffer solution before staining or directly into Giemsa stain, which is an aqueous stain. If thick films are to be stained later, they should be laked before storage. Potential problems with the preparation and staining of thick blood films can be seen in Table 7.3.

Thin Blood Films

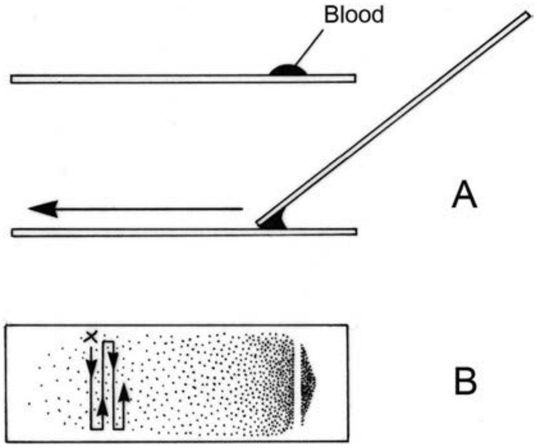
The thin blood film is routinely used for specific parasite identification, although the number of organisms per field is much reduced compared with the thick film. The thin film is prepared exactly as one used for a differential count

TABLE 7.3 Potential problems with thick blood film preparation and staining

Potential problem	Comments
Blood films flake off during staining	The blood film was not dry or did not dry evenly; the film was too thick; incorrect blood/anticoagulant ratio (excess EDTA); dirty or greasy slides (clean slides using gauze and 70% alcohol prior to use); blood kept in EDTA too long before film preparation
Inadequate staining	Blood film too thick; staining time incorrect; stain concentration incorrect
Potential for loss of organisms	Incorrect staining technique; blood film too old prior to staining; poor environmental conditions and/or incomplete drying

(Fig. 7.1). A well-prepared film is thick at one end and thin at the other (one layer of evenly distributed RBCs with no cell overlap). The thin, feathered end should be at least 2 cm long, and the film should occupy the central area of the slide, with free margins on both sides. The presence of long streamers of blood indicates that the slide used as a spreader was dirty or chipped. Streaks in the film are usually caused by dirt, and holes in the film indicate the presence of grease on the slide. After the film has air dried (do not apply heat), it may be stained. The necessity for fixation before staining will depend on the stain selected. Since Giemsa is an aqueous stain, laking of the RBCs occurs during the staining process. However, when Wright's stain is used, a fixing agent is incorporated into the stain, so that laking of the blood films must occur prior to staining. If using one of the rapid blood stains, read the package insert to confirm laking and/or fixation requirements for the thin and thick blood films.

Figure 7.1 Method for preparation of thin blood film. (A) Position of spreader slide; (B) well-prepared thin film. Arrows indicate the area of the slide (feather edge) used to observe accurate cell morphology. (Illustration by Sharon Belkin.)
doi:10.1128/9781555819002.ch7.f1



The instrument-prepared monolayer method or coverslip methods generally do not provide the best morphology for malarial parasites within the RBCs. However, the selection of a slide preparation method can be dictated by personal preference, since a malarial infection can be diagnosed from either type of slide. Potential problems with the preparation and staining of thin blood films can be seen in Table 7.4.

Combination Thick and Thin Blood Films (on the Same Slide)

In some instances (field surveys), it is helpful to prepare slides with both a thick and a thin film on the same slide. With this type of preparation, remember the following.

1. Sufficient time must be allowed for the thick portion of the smear to dry before staining.
2. If Giemsa stain is used, the thin film only must be fixed in absolute methanol before staining.

Combination Thick and Thin Blood Films (Can Be Stained as Either)

To prepare a slide containing blood that can be stained for either a thick or thin blood film, the following method was developed (Fig. 7.2). The specimen usually consists of fresh whole blood collected by finger stick or whole blood containing EDTA collected by venipuncture less than 1 h earlier.

Visually, the smear should consist of alternating thick and thin portions throughout the length of the glass slide.

TABLE 7.4 Potential problems with thin blood film preparation and staining

Potential problem	Comments
Blood films are too thick	Too much blood was used; no feathered edge; blood was pulled too quickly, spreader slide was held at the wrong angle (too high).
Blood films are too thin	Blood with a low hematocrit tends to spread too thin: let the blood settle or remove some plasma. Angle of the spreader slide was too low. Insufficient amount of blood for film preparation.
Blood films have rough edges; lack of or poor feather edge	Blood was in front of the spreader slide; uneven contact with spreader slide; spreader slide chipped; possible fibrin formation; slides greasy

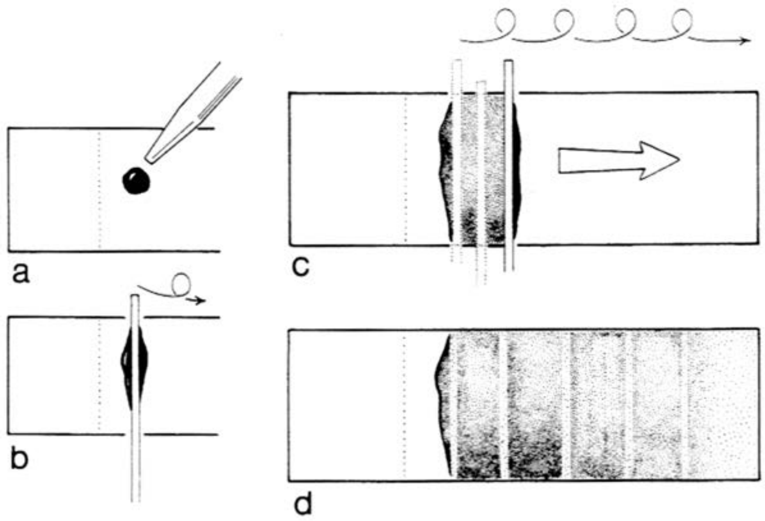


Figure 7.2 Method of thick-thin combination blood film preparation. (a) Position of the drop of EDTA-containing blood. (b) Position of the applicator stick in contact with blood and glass slide. (c) Rotation of the applicator stick. (d) Completed thick-thin combination blood film prior to staining. (Illustration by Sharon Belkin.) Reprinted from reference 9.
doi:10.1128/9781555819002.ch7.f2

One should be able to barely read newsprint through the wet or dry film. Also, the film itself should not have any clear areas or smudges, indicating that grease or fingerprints were on the glass.

Detailed Procedure

1. *Wear gloves when performing this procedure or preparing any blood films.*
2. The procedure depends on the source of the specimen.
   A. Blood from a finger puncture is not recommended, since the procedure does not lend itself to "stirring" to prevent fibrin strands.
   B. For blood from venipuncture, place a clean 1- by 3-in. glass microscope slide on a horizontal surface. Place a drop (30 to 40 μl) of blood onto one end of the slide about 0.5 in. from the end. Using an applicator stick lying across the glass slide and keeping the applicator in contact with the blood and glass, rotate (do not "roll") the stick in a circular motion while moving the stick down the glass slide to the opposite end. The appearance of the blood smear should be alternate thick and thin areas of blood that cover the entire slide. Immediately place the film over some small print and be sure that the print is just barely readable.
3. Allow the film to air dry horizontally and protected from dust for at least 30 min to 1 h. Do not attempt to speed the drying process by applying any type of heat, because the heat fixes the RBCs and they subsequently will not lyse (lake) in the staining process.
4. This slide can be stained as either a thick or thin blood film.
5. Label the slide appropriately.
6. If staining with Giemsa is delayed for more than 3 days or if the film is to be stained with Wright's stain, lyse the RBCs in the thick film by placing the slide in buffered water (pH 7.0 to 7.2) for 10 min, remove it from the water, and place it in a vertical position to air dry. Rapid stains are also acceptable.

Procedure Limitations

1. If the smears are prepared from anticoagulated blood that is more than 1 h old, the morphology of the parasites may not be typical and the film may wash off the slide during the staining procedure. **If a tube of blood containing EDTA cools to room temperature and the cap has been removed, several parasite changes can occur. The parasites within the RBCs will respond as if they were now inside the mosquito after being taken in with a blood meal. The morphology of these changes in the life cycle and within the RBCs can cause confusion when examining blood films prepared from this blood (3–8).**
   A. Stippling (Schüffner's dots) may not be visible.
   B. The male gametocyte (if present) may exflagellate and may resemble spirochetes (Fig. 7.3).
   C. The ookinetes of *Plasmodium* species other than *P. falciparum* may develop as if they were in the mosquito and may mimic the crescent-shaped gametocytes of *P. falciparum*.
2. Identification to species, particularly between *P. ovale* and *P. vivax* and between the ring forms of *P. falciparum* and *Babesia* spp., may be impossible without examining one of the slides stained as a thin blood film. Also, *Trypanosoma cruzi* trypomastigotes are frequently distorted in thick films.

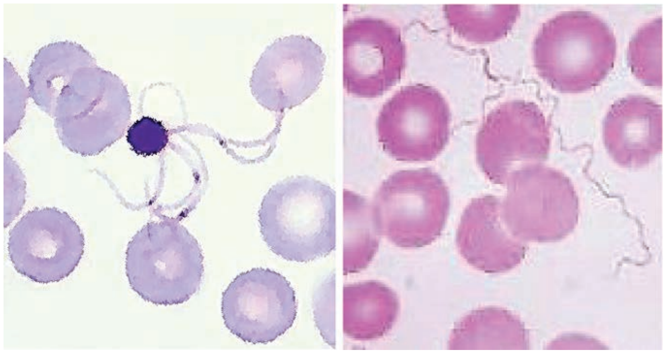


Figure 7.3 (Left) Photograph of exflagellation of the malarial microgametocyte. This may occur when anticoagulated blood is left standing at room temperature for some time prior to smear preparation. The life cycle of the parasite continues in the tube of blood as it would if the parasite had been ingested by the mosquito during a blood meal. (Right) *Borrelia* in blood. Note that the *Plasmodium* microgametes can resemble these organisms, particularly if they appear free in the background of the smear. doi:10.1128/9781555819002.ch7.f3

3. Excess stain deposition on the film may be confusing and make the detection of organisms difficult.
4. If the EDTA blood is 4 to 6 h old prior to blood films preparation, parasites are actually lost from the specimen; the parasitemia may be too low or represent a false negative.

Fixation of Thin Blood Films: Acetone Dip for Thick Blood Films

Thin-Film Fixation. Thin blood films must be completely dry before being fixed in absolute methanol (Giemsa staining). Drying times will be extended if the humidity is high; drying films as quickly as possible produces the best results. However, they should never be heated to decrease drying times; heat tends to produce distorted parasite and blood cell morphology. Absolute methanol tends to absorb moisture from the air, so that absolute methanol for fixation should not be reused from day to day but needs to be fresh daily. Do not store this fixative in a Coplin jar. Thin blood films should be fixed and stained within 24 h; deterioration may occur if the slides are held too long before being processed. However, the slides can be frozen for long-term storage. All thin blood films must be fixed prior to shipment to a reference laboratory; both these fixed slides and the original tube of blood should be sent to the reference laboratory. This assumes that the original laboratory has examined the blood films on site, as well.

If the thin blood films are fixed for too long, some of the morphologic details may be reduced or lost. If they are fixed for too short a time, the RBCs may be distorted or partially lysed and the thin film will not be uniformly stained. If the thin films are fixed in methanol that contains water, the RBCs will be distorted and there will appear to be “holes” in the thicker areas of the films. *As a reminder, do not reuse absolute methanol for fixation; discard after each use.*

Thick-Film Acetone “Quick Dip.” Although thick blood films are not fixed with absolute methanol, after the thick films are thoroughly dry, they can be dipped twice in acetone and allowed to dry before being stained. This extra step does not interfere with RBC lysis that occurs either prior to or during staining. The acetone “quick dip” makes the thick film less likely to fall off during staining and provides a cleaner background for microscopic examination.

Buffy Coat Blood Films

For patients with suspected malaria (negative thick and thin blood films), trypanosomiasis, filariasis, or leishmaniasis, concentration procedures increase the number of organisms recovered from blood specimens. The buffy coat containing WBCs and platelets, and the layer of RBCs just below the buffy coat layer, can be used to prepare thick and thin blood films. The sensitivity of this approach is greatly enhanced over that of the thick film. However, it is critical that the correct layer be sampled from the centrifuged blood.

L. donovani, trypanosomes, and *Histoplasma capsulatum* (a fungus with intracellular elements resembling those of *L. donovani*) are occasionally detected in the peripheral blood. The parasite or fungus is found in the large mononuclear cells in the buffy coat (a layer of white cells resulting from centrifugation of whole citrated blood). The nuclear material stains dark red-purple, and the cytoplasm is light blue (*L donovani*). *H. capsulatum* appears as a large dot of nuclear material (dark red-purple) surrounded by a clear halo area. Trypanosomes in the peripheral blood also concentrate with the buffy coat cells.

After centrifugation and aliquoting of the appropriate layers, some of the material can be examined as a wet mount; trypomastigotes and microfilariae may be seen as motile objects in the wet mount. After staining, *L. donovani* amastigotes may be found in the monocytes and *Plasmodium* parasites may be seen in the thick and thin films. Advantages and disadvantages of buffy coat films can be seen in Table 7.5.

Detailed Procedure

1. *Wear gloves when performing this procedure or preparing any blood films.*
2. Whole blood should be collected using EDTA anticoagulant. Although heparin can be used, if malaria films are to be prepared, EDTA is recommended.

TABLE 7.5 Advantages and disadvantages of the buffy coat films

Buffy coat film advantages	Buffy coat film disadvantages
The volume of blood is considerably larger than with both thick and thin films.	It takes some practice to remove the correct blood layers from the centrifuged blood in order to prepare thick and thin films.
More sensitive than thick films for diagnosis of malaria	Same potential problems may occur as with traditional thick or thin blood films.
Malaria pigment, which is phagocytized by the WBCs, may be seen more easily in the concentrated WBCs.	Some organisms and/or stages of parasites might be damaged due to high centrifugation; however, centrifugation at 500 × *g* for 15 to 20 min should not cause damage.
Detection of parasitemia is easier for larger stages such as schizonts and gametocytes.	Increased number of platelets may be confusing in terms of parasite differentiation.

3. Although capillary hematocrit tubes have been used in the past, the cutting and breaking of these tubes to remove the cells for film preparation is not considered a safe procedure and is *not* recommended. However, if you use a microhematocrit tube, the tube should be carefully scored and snapped at the buffy coat interface, and the white cells are prepared as a thin film. The tube can also be examined before removal of the buffy coat, at the low and high dry powers of the microscope. If trypanosomes are present, the motility may be observed in the buffy coat. Microfilarial motility would also be visible.
4. Using a capillary pipette, fill a Wintrobe tube with blood containing anticoagulant (EDTA is preferred), cap the tube, and centrifuge it for 30 min at 100 × *g*. Another option is to centrifuge the tube of anticoagulated blood at 100 × *g* for 15 min, transfer that buffy coat to another tube, and centrifuge the tube at 300 × *g* for 30 min. After centrifugation, the tube contains three layers: plasma on top, a layer of white cells (buffy coat), and the packed RBCs on the bottom.
5. Remove and discard most of the plasma above the buffy coat, leaving a small amount on top of the buffy coat layer. Then remove the remaining plasma, buffy coat, and the RBCs *right below the buffy coat*. Transfer this aliquot to a separate tube.
6. Examine the buffy coat directly for motile trypomastigotes and microfilariae by mixing 0.5 drop of saline with 1 drop of buffy coat sediment on a microscope slide. Add a coverslip, and examine at low power (×10 objective).
7. Mix the aliquot gently (avoid bubbles), and prepare thick and thin blood films on alcohol-cleaned slides.
8. Allow the films to air dry horizontally and protected from dust for at least 30 min to 1 h. Do not attempt to speed the drying process by applying any type of heat, because the heat fixes the RBCs and they subsequently will not lyse (lake) in the staining process for the thin films.
9. Label the slide appropriately.
10. If staining with Giemsa is delayed for more than 3 days or if the film is to be stained with Wright's stain, lyse the RBCs in the thick film by placing the slide in buffered water (pH 7.0 to 7.2) for 10 min, remove it from the water, and place it in a vertical position to air dry.

Staining Blood Films

For accurate identification of blood parasites, a laboratory should develop proficiency in the use of at least one good staining method (2, 9–11). It is better to select one method that will provide reproducible results than to use several on a hit-or-miss basis. Blood films should be stained as soon as possible, since prolonged storage may result in stain retention. Failure to stain positive malarial smears within a month may result in failure to demonstrate typical staining characteristics for individual species.

The most common stains are of two types. Wright's stain has the fixative in combination with the staining solution, so that both fixation and staining occur at the same time; therefore, the thick film must be laked before staining. Giemsa stain has the fixative and stain separate; therefore, the thin film must be fixed with absolute methanol before staining. If using one of the rapid blood stains, read the package insert to confirm laking and/or fixation requirements for the thin and thick blood films.

When slides are removed from either type of staining solution, they should be dried in a vertical position. After being air dried, they may be examined under oil immersion by placing the oil directly on the uncovered blood film. Remember, do not wipe the film to remove oil; lay slide oil side down on a paper towel to blot off the excess oil before storing the slide. If films are to be kept for a permanent record, they should be protected with a coverglass after being mounted in a medium such as Permount.

Note Blood films stained with any of the Romanowsky stains that have been mounted with Permount or other resinous mounting media are susceptible to fading of the basophilic elements and generalized loss of stain intensity. Hollander (12) has recommended the addition of 1% (by volume) 2,6-di-*t*-butyl-*p*-cresol (butylated hydroxytoluene [Sigma Chemical Co. catalog no. B1253]) to the mounting medium. Without the addition of this antioxidant, mounted stained smears eventually become pink; stains protected with this compound remain unchanged in color for many years.

Note Any slide that is protected by a coverglass and is going to be examined with an oil immersion lens must be covered by a no. 1 coverglass. If a no. 2 coverglass is used, the extra thickness may prevent the oil immersion lens from focusing properly.

Giemsa Stain

Giemsa stain is sold as a concentrated stock solution. Each new lot should be tested for optimal staining times before being used on patient specimens. If the blood cells appear to be adequately stained, the timing and stain dilution should be appropriate to demonstrate the presence of malarial and other parasites. Giemsa stain is also available as a powder for those who wish to make up their own stain. The use of prepared liquid stain or stain prepared from the powder depends on personal preference; there is apparently little difference between the two preparations. Directions for preparing the stain follow (4, 5, 9).

Note Quality control for blood film stains can be any negative or positive blood film; it is not necessary for the control slides to contain actual parasites. The actual patient slide being stained serves as its own control; if the WBCs look acceptable, any parasites present will also be acceptable in terms of morphology and staining colors. If the RBCs and WBCs stain correctly, any parasites present would also stain correctly with the same nuclear and cytoplasmic colors as the blood cells (see Fig. 7.4 to 7.8).

Reagents

Stock Giemsa Stain

Giemsa stain powder, certified (azure B type)	0.6 g
Methanol, absolute and certified neutral, acetone free	50 ml
Glycerin, neutral, certified	50 ml

1. Grind together small portions of stain and glycerin in a mortar, and collect mixtures in a 500- or 1,000-ml flask until all measured material is mixed.
2. Stopper the flask with a cotton plug, cover the plug with heavy paper, and place the flask in a 55 to 60°C water bath for 2 h. Make sure the water in the water bath is above the level of the stain. Shake gently at 30-min intervals.
3. After grinding the powder and glycerin in the mortar, use the 50 ml of methyl alcohol to wash the last bit of stain from the mortar; then pour the alcohol into a small, airtight bottle.
4. Remove the glycerin-stain powder mixture from the water bath, and allow it to cool to room temperature. Add alcohol washing from the mortar, and shake well.
5. Before use, filter through Whatman no. 1 paper into a brown bottle. Although the stain can be used immediately, it is better to let it stand for 2 to 3 weeks with intermittent shaking.
6. Label and store protected from light; the shelf life is 36 months, provided that results are within quality control guidelines.

Note The stock stain is stable for many years; however, it must be protected from moisture. The staining reaction is oxidative; any oxygen in water will initiate the staining reaction and destroy the stock stain. This is why the aqueous working solution of stock stain is good only for 1 day.

10% Stock Solution of Triton X-100

Triton X-100	10 ml
Distilled water	90 ml

Mix thoroughly, and store at room temperature. This solution will keep indefinitely if kept tightly stoppered.

Stock Buffers

Disodium Phosphate (Dibasic)

Na_2HPO_4, anhydrous	9.5 g
Distilled water	1,000 ml

Monosodium Phosphate (Monobasic)

NaH_2PO_4 H_2O	9.2 g
Distilled water	1,000 ml

Phosphate-Buffered Water (Table 7.6)

Triton-Phosphate Buffer

0.01% Triton-Buffered Water

Stock 10% aqueous Triton X-100	1 ml
Phosphate buffer	1,000 ml

0.1% Triton-Buffered Water

Stock 10% aqueous Triton X-100	10 ml
Phosphate buffer	1,000 ml

For thin blood films or a combination of thin and thick blood films, use 0.01% Triton-buffered water; for thick blood films, use 0.1% Triton-buffered water.

Liquid

The commercial liquid stain or the stock solution prepared from powder should be diluted approximately the same amount to prepare the working stain solution. Stock Giemsa liquid stain is diluted 1:10 with buffer for thin blood films; for thick films, a dilution of up to 1:50 may be used. Some people prefer to stain both thick and thin smears for a longer

TABLE 7.6 Phosphate buffer solutions

	Amount (ml) of:		
pH	Na_2HPO_4 (9.5 g/liter, anhydrous)	$NaH_2PO_4 \cdot H_2O$ (9.5 g/liter)	Distilled water
6.6	37.5	62.5	900
6.8	49.6	50.4	900
7.0	61.1	38.9	900
7.2	72.0	28.0	900

period in a more dilute solution. Phosphate buffer used in dilution of the stock stain should be neutral or slightly alkaline. Phosphate buffer solution may be used to obtain the right pH (Table 7.6). In some laboratories, tap water has a satisfactory pH and may be used for the entire staining procedure and the final rinse. Some workers recommend using pH 6.8 to emphasize Schüffner's dots.

Procedure for Staining Thin Films

1. Fix blood films in absolute methyl alcohol (acetone free) for 1 min.
2. Allow the slides to air dry.
3. Immerse the slides in a solution of 1 part Giemsa stock (commercial liquid stain or stock prepared from powder) to 10 to 50 parts phosphate buffer (pH 7.0 to 7.2). Stain for 10 to 60 min. *Fresh working stain should be prepared from stock solution each day.*

   Note A good general rule for stain dilution versus staining time is as follows. If the dilution is 1:20, stain for 20 min; if the dilution is 1:30, stain for 30 min, etc. However, a series of stain dilutions and staining times should be tried to determine the best dilution and time for each batch of stock stain.
4. Dip the slides briefly in phosphate-buffered water, or rinse under gently running tap water.

   Note Excessive washing will decolorize the film.
5. Drain thoroughly in a vertical position, and allow to air dry. The bottom of the slide should be wiped to remove excess stain before drying.

Procedure for Staining Thick Films

The procedure to be followed for thick films is the same as for thin films, except that the first two steps are omitted. If the slide has a thick film at one end and a thin film at the other, fix only the thin portion and then stain both parts of the film simultaneously. Normally, a stain/buffer dilution of 1:50 (vol/vol) for a staining time of 50 min is recommended for thick films. The longer staining time seems to give better results than do the shorter times that can be used for the thin film.

Results

Giemsa stain colors the components of blood as follows: RBCs, pale red; nuclei of WBCs, purple with pale purple cytoplasm; eosinophilic granules, bright purple-red; and neutrophilic granules, deep pink-purple. If malaria parasites are present, the cytoplasm stains blue and the nuclear material stains red to purple-red. Schüffner's dots and other inclusions in the RBCs will stain red (Fig. 7.4). *However*, as seen in Fig. 7.4, there can be tremendous variation in color. Nuclear and cytoplasmic staining characteristics of the other blood parasites such as *Babesia* spp., trypanosomes, and leishmaniae are like those of the malaria parasites (Fig. 7.5 to 7.8). While the sheath of microfilariae may not always stain with Giemsa, the nuclei within the microfilaria itself stain blue to purple. The sheath of *Brugia malayi* will tend to stain pink, while the sheath of *Wuchereria bancrofti* may not stain with Giemsa (Fig. 7.9).

Wright's Stain

Wright's stain is available commercially in liquid form, ready to use, and also as a powder which must be dissolved in anhydrous, acetone-free methyl alcohol before use. Directions for preparing the stain follow.

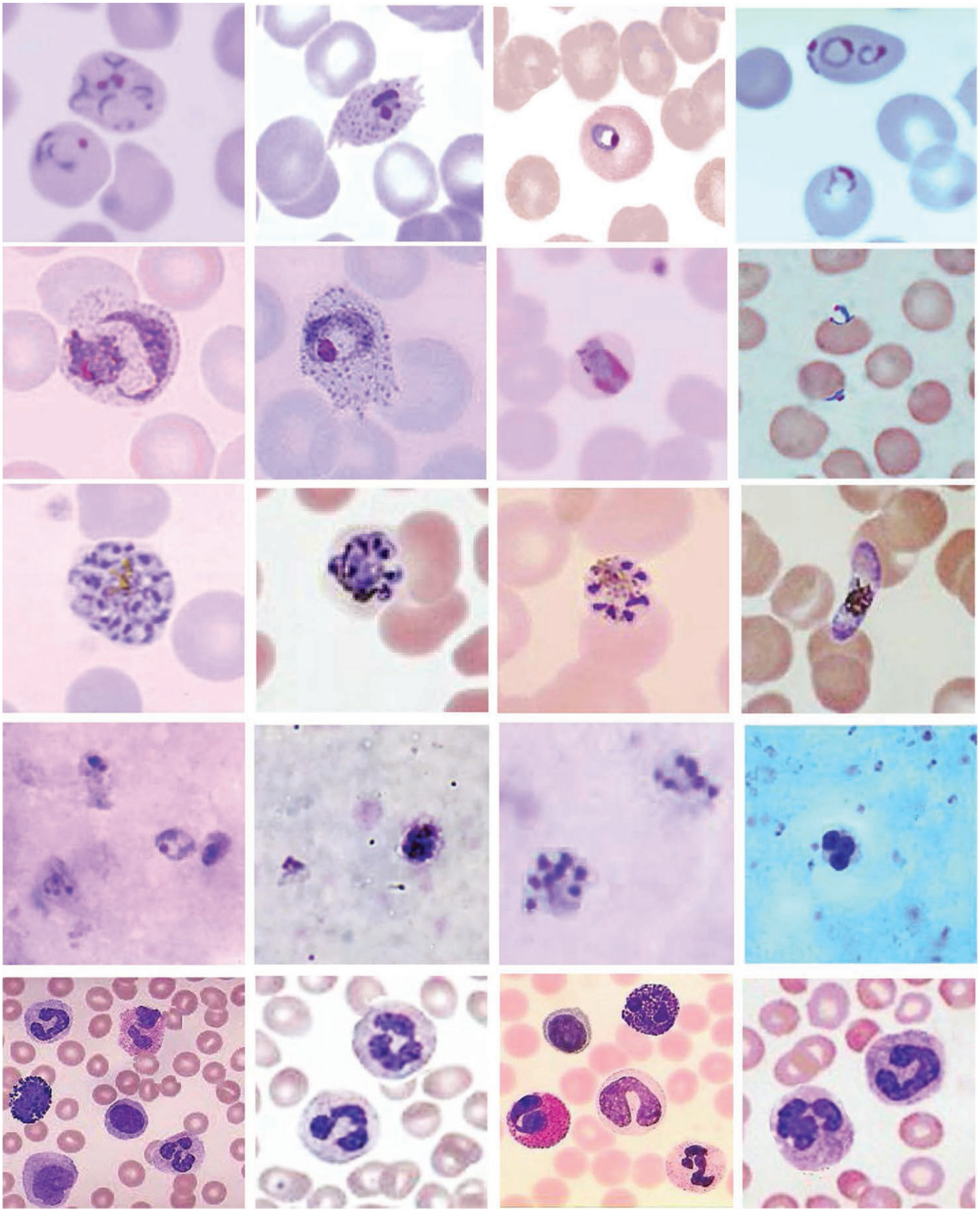
Reagent

Wright's Stain

Wright's stain	0.9 g
Methanol, absolute and certified neutral, acetone free	500.0 ml

1. Grind 0.9 g of Wright's stain powder with 10 to 15 ml of methanol (anhydrous, acetone free) in a clean mortar. Gradually add methanol while grinding. As the dye is dissolved in the methanol, pour that solution off and add more methanol to the mortar. Repeat this process until the entire 500 ml of methanol has been used.
2. Store the stain in a tightly stoppered glass bottle at room temperature. Shake the bottle several times daily for at least 5 days.
3. Allow the precipitate to settle, and pour off some of the supernatant fluid into dropping bottles for use. If the stock solution has been disturbed, the supernatant fluid can be filtered through Whatman no. 1 paper into a brown bottle.
4. Label and store protected from light; the shelf life is 36 months, provided that results are within quality control guidelines.

The staining procedure requires phosphate buffer solutions (see directions in the preceding discussion of Giemsa stain); the pH required for Wright's stain is 6.6 to 6.8.



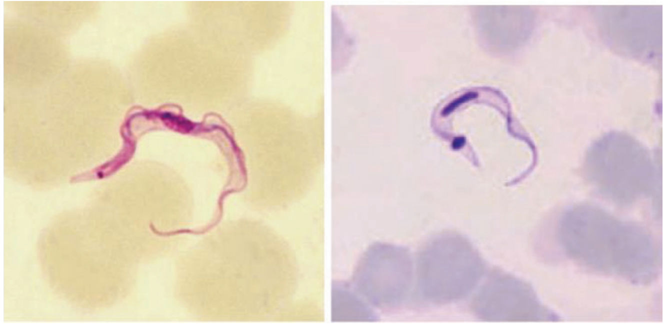


Figure 7.5 Trypomastigotes. (Left) *Trypanosoma brucei gambiense*. (Right) *Trypanosoma cruzi* (note the undulating membrane on both trypomastigotes and the larger kinetoplast in *T. cruzi*). doi:10.1128/9781555819002.ch7.f5

Procedure for Staining Thin Films

Since Wright's stain contains alcohol, the slides do not require fixation before staining.

1. Place a slide on a rack in a horizontal, level position, and cover the surface with stain.
2. Count the number of drops of stain needed to cover the surface. Let stand for 1 to 3 min (the optimal staining time varies with each batch of stain).
3. Add an equal number of drops of phosphate-buffered water to the slide; mix the stain and buffer by blowing on the surface of the fluid.
4. After 4 to 8 min, flood the stain from the slide with phosphate buffer. Do not pour the stain off before washing. If you do, a precipitate will be deposited on the slide.
5. Wipe the bottom of the slide to remove excess stain.
6. Allow the slide to drain and air dry.

Procedure for Staining Thick Films

Thick films stained with Wright's stain are usually inferior to those stained with Giemsa solution. Great care should

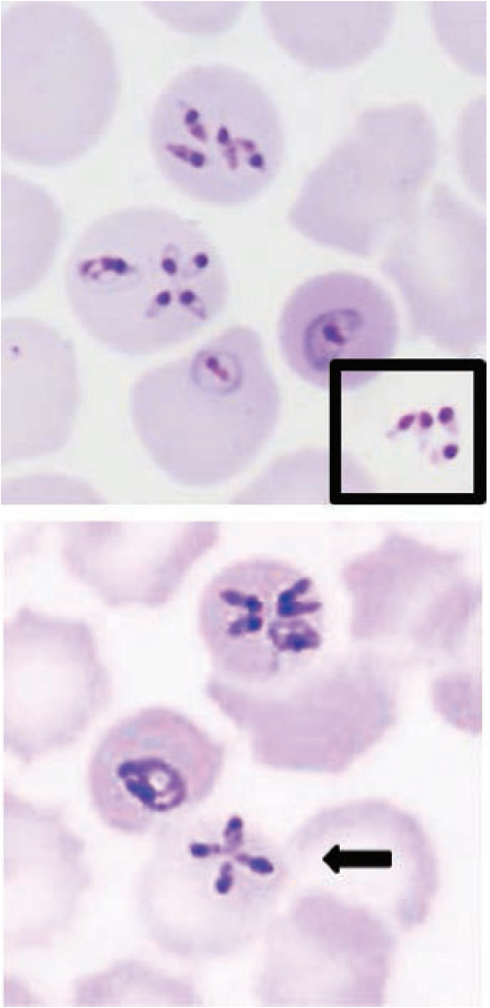


Figure 7.6 *Babesia* sp. (Top) Note some of the ring forms are outside the RBCs (box, rare to see in *Plasmodium* infections). (Bottom) Note the numerous ring forms per RBC; also note the Maltese cross ring formation in the lowermost RBC (arrow). doi:10.1128/9781555819002.ch7.f6

Figure 7.4 *Plasmodium* spp. seen in stained thin blood films. (Left column from top to bottom) *Plasmodium vivax*: (1) developing ring (note the enlarged RBC, lack of Schüffner's dots [EDTA blood], and ameboid rings); (2) developing trophozoite (note the enlarged RBC and Schüffner's dots); (3) mature schizont with approximately 16 merozoites; (4) thick blood film showing developing ring forms; (5) normal WBCs (including polymorphonuclear leukocytes [PMNs], eosinophil, basophil, monocyte, and lymphocyte). (Second column from top to bottom) *Plasmodium ovale*: (1) young ring form (note the nonameboid ring and Schüffner's dots that appear earlier than in rings of *P. vivax*); (2) developing trophozoites (note Schüffner's dots and fimbriated edges of the infected RBCs); (3) maturing schizont containing merozoites and malarial pigment; (4) thick blood film showing developing schizont; (5) normal PMNs. (Third column from top to bottom) *Plasmodium malariae*: (1) young ring (note the normal-sized RBC and no stippling); (2) developing trophozoite (note the normal-sized RBC and "band form" configuration of the trophozoite); (3) mature schizont (note the size of the RBC) containing approximately eight merozoites and brownish malarial pigment; (4) thick blood film with mature schizonts containing developing merozoites; (5) normal WBCs (including PMN, eosinophil, basophil, monocyte and lymphocyte). (Last column from top to bottom) *Plasmodium falciparum*: (1) typical ring forms (note appliqué form at the side of the RBC and multiple rings/cell); (2) typical ring forms (note the ring appearing to be partially outside the RBC—typical for *P. falciparum*); (3) crescent-shaped gametocyte; (4) thick blood film showing many ring forms; (5) typical PMNs. doi:10.1128/9781555819002.ch7.f4

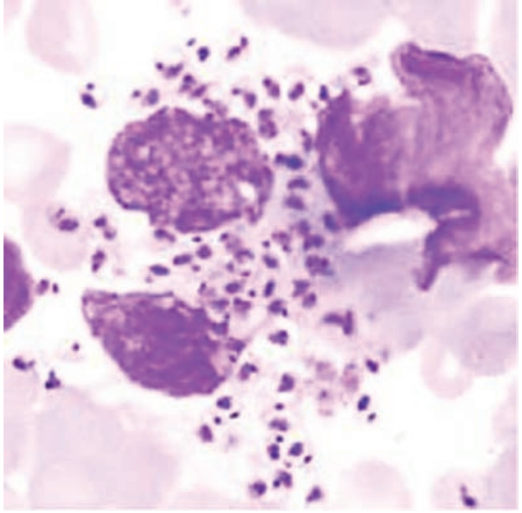


Figure 7.7 *Leishmania donovani* in an impression smear. Note the numerous small amastigotes containing a nucleus and bar-shaped primitive flagellum. doi:10.1128/9781555819002.ch7.f7

also be taken to avoid excess stain precipitate on the slide during the final rinse. Before being stained, thick films must be laked in distilled water (to rupture and remove RBCs) and air dried. The staining procedure is the same as for thin films, but the staining time is usually somewhat longer and must be determined for each batch of stain.

Results

Wright's stain colors blood components as follows: RBCs, light tan, reddish, or buff; nuclei of WBCs, bright blue with contrasting light cytoplasm; eosinophilic granules, bright red; and neutrophilic granules, pink or light purple.

If malaria parasites are present, the cytoplasm stains pale blue and the nuclear material stains red. Schüffner's dots and other inclusions in the RBCs usually do not stain or stain very pale with Wright's stain. Nuclear and cytoplasmic staining characteristics of the other blood parasites such as *Babesia* spp., trypanosomes, and leishmaniae are like those of the malaria parasites. While the sheath of microfilariae may not always stain with Wright's stain, the nuclei within the microfilaria itself stain pale to dark blue.

General Notes on Staining Procedures

1. When large numbers of slides are being processed, remember that dry films should be stored in dust-free containers before staining, to protect fresh smears from insects.
2. If the slides cannot be stained within 48 h, thin films should be fixed in methyl alcohol and thick films should be laked in distilled water before storage (some users prefer buffered water at pH 6.8 to 7.2). Rapid stains can also be used. Parasites will stain like the WBCs.
3. Slides should be stored at reasonably low temperatures (below 80°F [ca. 27°C]) before being stained. If the slides are exposed to high temperatures, thick films that have not been laked will become heat fixed; hemoglobin will remain fixed in the RBCs, and heavy stain retention will then prevent parasite identification. Thin films also stain poorly after exposure to high temperatures.
4. Fresh working stain should be prepared just before use. If a large number of thick films are being laked during staining, the stain should be changed after 50 slides because of the accumulation of excess hemoglobin.

Proper Examination of Thin and Thick Blood Films

Thin Blood Films

In any examination of thin blood films for parasitic organisms, the initial screen should be carried out with the low-power objective of a microscope. Microfilariae may be missed if the entire thin film is not examined. Microfilariae are rarely present in large numbers, and frequently only a few organisms occur in each thin-film preparation. Microfilariae are commonly found at the edges of the thin film or at the feathered end of the film, because they are carried to these sites during the process of spreading the blood. The feathered end of the film where the RBCs are drawn out into one single, distinctive layer of cells should be examined for the presence of malaria parasites and trypanosomes. In these areas, the morphology and size of the infected RBCs are most clearly seen.

Depending on the training and experience of the microscopist, examination of the thin film usually takes 5 to 10 min or less (200 to 300 oil immersion fields) at a magnification of ×1,000. Although some people use a 50× or 60× oil immersion objective to screen stained blood films, there is some concern that small parasites such as plasmodia, *Babesia* spp., or *L. donovani* may be missed at this smaller total magnification (×500 or ×600) compared with the ×1,000 total magnification obtained with the more traditional 100× oil immersion objective. Because people tend to scan blood films at different rates, it is important to examine a minimum number of fields, regardless of the time it takes to perform this procedure. If something suspicious has been seen in the thick film, the number of fields examined on the thin film is often considerably more than 200 to 300. The request for blood film examination should always be considered a STAT procedure, with all reports (negative as well as positive) being reported immediately to the physician as soon as

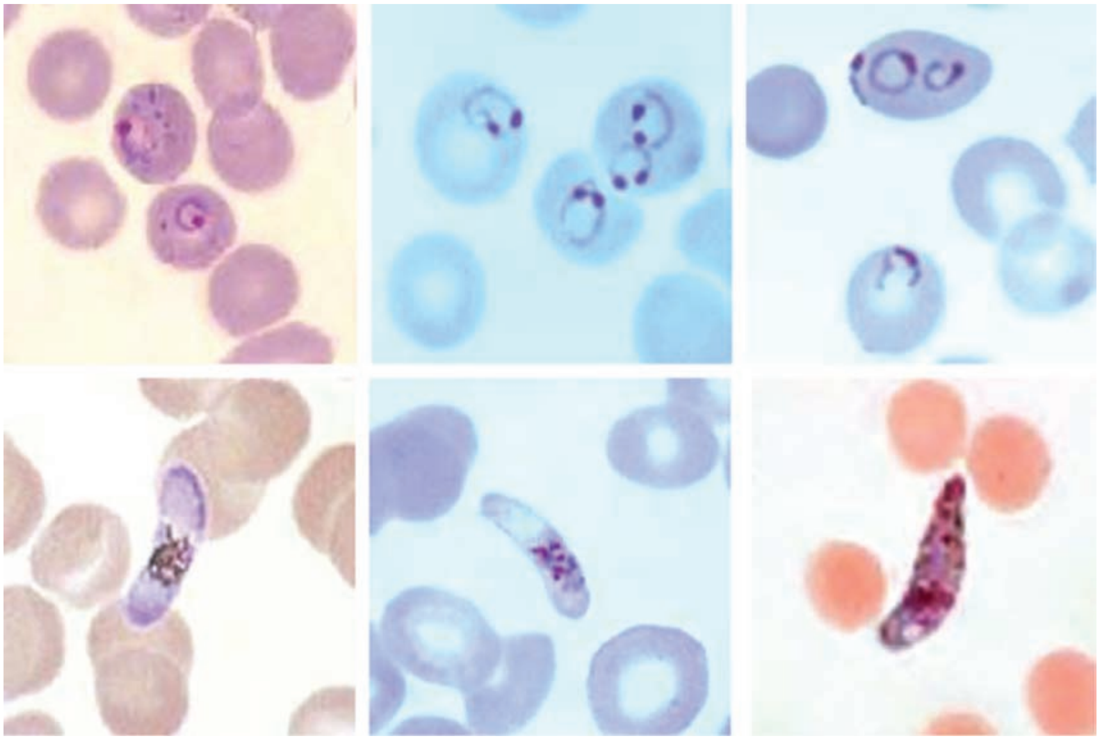


Figure 7.8 *Plasmodium falciparum*. (Upper row) Note the ring forms (multiple rings per RBC and presence of the "headphone" ring configuration). The photograph at the far right is a good mimic of *Babesia* organisms, but the rings are not quite as pleomorphic as in *Babesia* spp. (Lower row) Two examples of *P. falciparum* gametocytes, two of which appear to be outside the RBC. However, although the RBC membrane is not visible, the gametocyte is intracellular. The image on the far right is an ookinete from the mosquito cycle; this can occur within the EDTA blood specimen if the blood cools and is left standing with the cap removed. In the cooled, aerated blood, the parasites begin the cycle that normally occurs within the mosquito. This artifact can easily be confused with the crescent-shaped gametocyte. doi:10.1128/9781555819002.ch7.f8

possible. Appropriate governmental agencies (local, state, and federal) should be notified within a reasonable time frame in accordance with guidelines and laws.

Diagnostic problems with the use of automated differential instruments have been reported (13, 14). Both malaria and *Babesia* light infections (like those seen with travelers with no prior exposure to malaria) were missed with these instruments, and therapy was delayed. Although these instruments are not designed to detect intracellular blood parasites, the inability of the automated systems to discriminate between uninfected RBCs and those infected with parasites may pose serious diagnostic problems.

Thick Blood Films

In the preparation of a thick blood film, the greatest concentration of blood cells will be in the center of the film. A search for parasitic organisms should be carried out initially at low magnification to detect microfilariae more readily. Examination of a thick film usually requires 3 to 5 min (approximately 100 oil immersion fields). Search for malarial organisms and trypanosomes is best done under oil immersion (total magnification, ×1,000). Intact RBCs are frequently seen at the very periphery of the thick film; such cells, if infected, may prove useful in malaria diagnosis, since they may demonstrate the characteristic morphology necessary to identify the organisms to the species level.

Determination of Parasitemia

It is important to report the level of parasitemia when blood films are reviewed and found to be positive for malaria parasites. Because of the potential for drug resistance in some of the *Plasmodium* species, particularly *P. falciparum* and *P. vivax*, it is important that every positive smear be assessed and the parasitemia reported exactly the same way on follow-up specimens as on the initial specimen. This allows the parasitemia to be monitored after therapy has been initiated. In cases where the patient is hospitalized, monitoring should be performed at 24, 48,

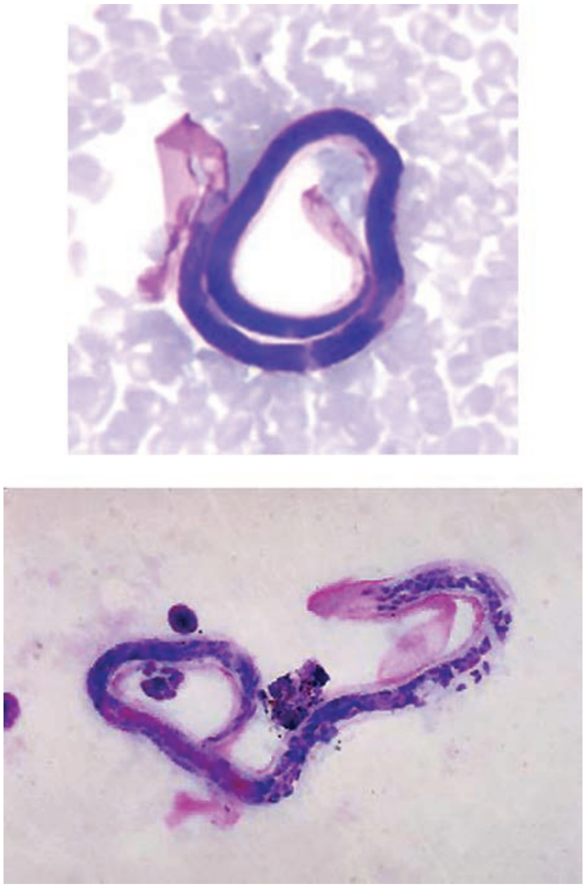


Figure 7.9 (Upper) *Brugia malayi* microfilaria on a blood film stained with Giemsa stain. Note the presence of the sheath (stains pink with Giemsa stain). (Lower) *Brugia malayi* microfilaria on a thick blood film stained with Giemsa stain (note some of the individual nuclei are visible). (Images courtesy of the CDC Public Health Image Library; lower, photograph by Dr. Mae Melvin.) doi:10.1128/9781555819002.ch7.f9

and 72 h after initiating therapy. Generally, the parasitemia drops very quickly within the first 2 h; however, in cases of drug resistance, the level may not decrease but actually may increase over time.

Malarial infections should be reported as the percentage of infected RBCs per 100 RBCs counted (0.5%, 1.0%, etc.) (3). Considering the low parasitemia frequently seen in patients within the United States, several hundred RBCs may have to be counted to arrive at an accurate count and determination of the percentage. The thin blood film must be used for this approach.

Another approach is to count the number of parasites per 100 WBCs on the smear. Either the thick or thin film can be used for this purpose. This figure can be converted to the number of parasites per microliter of blood; divide the number of parasites per 100 WBCs by 100, and multiply that figure by the number of WBCs per microliter of blood. Depending on the parasitemia, 200 or more WBCs may have to be counted, so the denominator may vary (it may be 200 or even more). In this case, blood for both the peripheral smears and cell counts must be collected at the same time.

It is critical that the same reporting method be used consistently for every subsequent set of blood films so that the parasitemia can be tracked for decrease or possible increase, indicating resistance. Also, remember that drug resistance may not become evident for a few days. The parasitemia may initially appear to decline but may then begin to increase after several days. Therefore, it is very important that patient parasitemia be monitored, particularly if an infection with *P. falciparum* has been diagnosed. Drug resistance has also been reported in *P. vivax* cases; mixed infections are also much more common than suspected.

Although therapy for *Babesia* infections is usually quite effective, calculation of parasitemia for positive *Babesia* infections is highly recommended. Babesiosis usually results in self-cure or cure after administration of azithromycin plus atovaquone or clindamycin plus quinine. Although certain individuals who are severely immunocompromised may not respond to these drug regimens, there has been no reported evidence that treatment failure is due to drug-resistant strains of *Babesia microti*. Preliminary studies indicate that reduction of pathogen loads and WBC inactivation within blood components using riboflavin and ultraviolet light have been used as an alternative for gamma-irradiation for *B. microti*, *Babesia divergens*, *T. cruz*i, HIV, and bacteria (15).

Diagnosis of Malaria: Review of Alternatives to Conventional Microscopy

It is well known that malaria causes significant morbidity and mortality worldwide, including in countries where imported cases are seen. In many developing countries where malaria is highly endemic, diagnostic testing is often inadequate or unavailable due to a lack of trained personnel or funds or both. Although microscopic examination of stained thick and thin blood films remains the standard of practice, this approach is time-consuming, is based on the need for a great deal of expertise in microscopic morphology, and requires the purchase and maintenance of expensive equipment. Rapid diagnostic tests (RDTs) offer great potential to improve the diagnosis of malaria, particularly in remote areas (World Health Organization. 2012. Malaria rapid diagnostic test performance. Summary results of WHO product testing of malaria RDTs: Round 1–4 [2008–2012]; http://www.who.int/malaria/publications/rdtmalaria_ [accessed 6/20/2013]). This provides very comprehensive lists of product testing to date. There are a number of new approaches to the diagnosis of malaria, including the use of fluorescent stains (QBC),

TABLE 7.7 Parasitemia determined from conventional light microscopy: clinical correlation[a]

Parasitemia (%)	No. of parasites/μl	Clinical correlation
0.0001–0.0004	5–20	Number of organisms that are required for a positive thick film (sensitivity) Examination of 100 thick-blood-film fields (0.25 μl) may miss up to 20% of infections (sensitivity, 80–90%); at least 300 fields should be examined before reporting a negative result. Examination of 100 thin-blood-film fields (0.005 μl); at least 300 should be examined before reporting a negative result; **both** thick and thin blood films should be examined for every specimen submitted for a suspect malaria case. **One set (thick plus thin blood films) of negative blood films does not rule out a malaria infection.**
0.002	100	Naive patients may be symptomatic below this level; remember emergency room patients (travelers with no prior exposure to malaria).
0.1	5,000	BinaxNOW rapid malaria test (maximum sensitivity at this level); sensitivity decreases as parasitemia decreases
0.2	10,000	Level above which immune patients will exhibit symptoms
2	100,000	Maximum parasitemia of *P. vivax* and *P. ovale* (which infect young RBCs only)
2–5	100,000–250,000	Hyperparasitemia, severe malaria[b]; increased mortality
10	500,000	Exchange transfusion may be considered; high mortality

[a] Adapted from reference 24.

[b] World Health Organization criteria for severe malaria are parasitemia of >10,000/μl and severe anemia (hemoglobin, >5 g/liter). Prognosis is poor if 0.20% of parasites are pigment-containing trophozoites and schizonts and/or if >5% of neutrophils contain visible pigment.

dipstick antigen detection of histidine-rich protein 2 (HRP2), parasite lactate dehydrogenase (pLDH), and Flow anti-pLDH *Plasmodium* monoclonal antibodies), PCR, and automated blood cell analyzers. Parasitemia and its clinical correlates are given in Table 7.7, while some of the testing options for malaria can be found in Table 7.8. Testing options for some of the other blood parasites can be seen in Table 7.9.

Histidine-rich protein 2 of *P. falciparum* (PfHRP2) is a water-soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum*, expressed on the RBC membrane surface, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy. Many RDTs are based on the detection of PfHRP2, but reports from field tests have questioned their sensitivity and reliability. However, the variability in the results of PfHRP2-based RDTs may be related to the variability in the target antigen (16). This hypothesis was tested by examining the genetic diversity of PfHRP2, which includes numerous amino acid repeats, in 75 *P. falciparum* lines and isolates originating from 19 countries and testing a subset of parasites by use of two PfHRP2-based RDTs. There is extensive diversity in PfHRP2 sequences, both within and between countries. Logistic regression analysis indicated that two types of repeats were predictive of RDT detection sensitivity (87.5% accuracy), with predictions suggesting that only 84% of *P. falciparum* parasites in the Asia-Pacific region are likely to be detected at densities of ≤250 parasites/μl. Data also indicate that PfHRP3 may play a role in the performance of PfHRP2-based RDTs. These findings provide an alternative explanation for the variable sensitivity in field tests of malaria RDTs that is not due to the quality of the RDTs (16).

The persistence of parasite HRP2 in the circulation after parasite clearance has been considered a drawback for RDTs targeting HRP2 and a major cause of false-positive results. In one study when PCR was used as the gold standard rather than microscopy, the high rate of RDT false-positive parasitemia results in comparison with microscopy was shown to predominantly represent cases that had a parasite density below the threshold for detection by microscopy (17). Despite the generally low disease-endemic prevalence of malaria in the area, there was a high prevalence of chronic infections with low, fluctuating parasite densities that were better detected by RDT. In areas known to have low-density parasitemias, RDTs targeting HRP2 may increase the diagnostic sensitivity in comparison with microscopy. While microscopy remains the standard for comparison of the diagnostic accuracy for malaria, the limitations of microscopy, and the possibility that RDTs may have superior accuracy in some circumstances, should be taken into account when interpreting the results of diagnostic trials.

To determine the accuracy of RDTs for ruling out malaria in nonimmune travelers returning from areas where malaria is endemic, 21 studies and 5,747 individuals were surveyed from the published literature (18). Diagnostic accuracy studies of nonimmune individuals with suspected malaria were included if they compared rapid tests with expert microscopic examination or PCR tests. The authors concluded that RDTs for malaria may be a useful diagnostic adjunct to microscopy in centers without major expertise in tropical medicine. Initial decisions on treatment initiation and choice of antimalarial drugs can be based on travel history and posttest probabilities after rapid testing. However, expert microscopy is still required for species identification and confirmation.

TABLE 7.8 Rapid diagnostic test kits for detection of antigen of *Plasmodium* species that are currently available for sale[a]

Manufacturer	Product name	Catalog no.	Malaria species[b]	Target antigen[c]	Test format
Alere, FDA approved	BinaxNOW Malaria	Not stated	Pf/Pan	Pf HRP2, LDH	Lateral flow
Alere	Clearview Malaria Pf test	Not stated	Pf	Pf HRP2, aldolase	Lateral flow
Alere	Clearview Malaria Combo	Not stated	Pf/Pan	Pf HRP2, aldolase	Lateral flow
Core Diagnostics	Core Malaria Pf	Not stated	Pf	Pf HRP2	Lateral flow
Core Diagnostics	Core Malaria Pan-Pf	Not stated	Pf/Pan	LDH, Pf HRP2	Lateral flow
Core Diagnostics	Core Malaria Pv-Pf	Not stated	Pf/Pv	LDH	Lateral flow
Core Diagnostics	Core Malaria Pan-Pv-Pf	Not stated	Pf/Pv/Pan	LDH, Pf HRP2	Lateral flow
Diagnostic Automation Cortez Diagnostics	Malaria (Pf/Vivax)	172110-25	Pf/Pv	Pf HRP2, Pv LDH	Lateral flow
Diagnostic Automation Cortez Diagnostics	Malaria (Pan-LDH)	172120-25	Pan	LDH	Lateral flow
Human GmbH	Hexagon Malaria Combi	58054	Pf/Pan	Pf HRP2, aldolase	Lateral flow
Immunospec	Malaria Pf	R14-537	Pf/Pan	LDH	Lateral flow WB
Immunospec	Malaria Pf	R14-530	Pf	LDH	Lateral flow WB
Immunospec	Malaria Pf/vivax Cassette	R14-538	Pf/Pv	LDH	Lateral flow WB
Immunospec	PF Malaria Rapid test	R6-537	Pf	LDH	Lateral flow WB
Omega Diagnostics	Visitect Malaria Pf	OD026	Pf	Pf HRP2	Lateral flow
Omega Diagnostics	Visitect Malaria Combo Pan, Pf	OD206	Pf/Pan	Pf HRP2, LDH	Lateral flow
Omega Diagnostics	Paracheck Pf Dipstick	Not stated	Pf	Pf HRP2	Solid phase dipstick
Premier Medical Corp.	First Response Malaria Pf, Pv, Ag	106FRS48	Pf/Pan	LDH	Lateral flow strip
Premier Medical Corp.	First Response Malaria Ag	112FRC30	Pan	LDH	Lateral flow card
Premier Medical Corp.	First Response Malaria Ag	113FRC30	Pf	Pf HRP2	Lateral flow card
Premier Medical Corp.	First Response Malaria Ag	115FRS30	Pf/Pan	LDH, Pf HRP2	Lateral flow strip
Premier Medical Corp.	First Response Malaria Ag Combo Rapid	116FRC30	Pf/Pan	LDH, Pf HRP2	Lateral flow card
Span Diagnostics	ParaHIT-f	25977	Pf	Pf HRP2	Solid phase dipstick
Span Diagnostics	ParaHIT-f	25978	Pf	Pf HRP2	Lateral flow device
Span Diagnostics	ParaHIT Total	25988	Pf/Pan	Pf HRP2, aldolase	Solid phase dipstick
Span Diagnostics	ParaHIT Total	25989	Pf/Pan	Pf HRP2, aldolase	Lateral flow
Standard Diagnostics	SD BIOLINE Malaria Ag	05FK40	Pf/Pan	Pf HRP2, LDH	Lateral flow
Standard Diagnostics	SD BIOLINE Malaria Ag Pf	05FK50	Pf	Pf HRP2	Lateral flow
Standard Diagnostics	SD BIOLINE Malaria Ag Pf/Pan	05FK60	Pf/Pan	Pf HRP2, LDH	Lateral flow
Zephyr Biomedicals (Tulip Group)	PARABANK	50301010 50301025	Pan	LDH	Lateral flow device
Zephyr Biomedicals (Tulip Group)	PARABANK	50302010 50302025	Pan	LDH	Lateral flow dipstick
Zephyr Biomedicals (Tulip Group)	PARASCREEN	50310010 50310025	Pan	Pf HRP2, LDH	Lateral flow device
Zephyr Biomedicals (Tulip Group)	FALCIVAX	50300010 50300025	Pf, Pv	Pf HRP2, Pv LDH	Lateral flow device
Zephyr Biomedicals (Tulip Group)	PARAMAX-3	50320010 50320025	Pf/Pv/Pan	Pf HRP2, Pv LDH, Pan LDH	Lateral flow device
Zephyr Biomedicals (Tulip Group)	MALASCAN	50402001 50402010 50402025	Pf/Pan	Pf HRP2, Pan LDH	Lateral flow device

[a] List as of February, 2013. This is not a complete listing of available products, but reflects information that was readily available. Products for the detection of antibody are not included. Check company websites for product details.

[b] Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*; Pan, panspecific antigen common to all *Plasmodium* spp.

[c] HRP2, histidine-rich protein 2; LDH, lactate dehydrogenase.

TABLE 7.9 Summary of commercially available kits for immunodetection of blood parasite antigens or antibodies (other than *Plasmodium* sp.)[a]

Organism and kit name	Manufacturer and/or distributor	Type of test	Sensitivity, specificity (%)	Comments
Protozoa				
Leishmania (visceral)				
Leishmania RapiDip InstaTest	Cortez Diagnostics	Rapid	100, NA	Antibody—visceral
	Immunetics	IB		Antibody
Kalazar Detect	InBios	EIA, rapid		Antibody
Leishmania ELISA	IVD Research	EIA		Antibody
Trypanosoma cruzi (Chagas' disease)				
T. cruzi IgG CELISA	Cellabs	EIA	100, 100	Antibody
Chagas' EIA	Chemicon Intl.	EIA		Antibody
Chagas'	Hemagen	EIA	100, 100	Antibody
Trypanosoma Detect	InBios	EIA, rapid	100, 100	Antibody
Chagas' ELISA	IVD Research	EIA	100, 93	Antibody
Helminths				
Wuchereria bancrofti				
Binax NOW Filariasis	Alere	Rapid	100, 96	Antigen
Filariasis CELISA	Cellabs	EIA		Antigen
Filariasis	TropBio	EIA		Antigen

[a] Check websites for additional information. Abbreviations: EIA, enzyme immunoassay; IB, immunoblot; IgG, immunoglobulin G; NA, not available.

Diagnostic accuracy of RDTs for self-diagnosis was variable, and requires improvements before widespread acceptance (19). RDTs have limitations that include the difficulty in detecting mixed infections, all species of *Plasmodium*, and infections at low concentrations of parasites, along with an inability to monitor response to therapy. Also, if the result is negative, microscopy is still mandatory. Therefore RDTs do not eliminate the need to examine thick and thin blood films. The maintenance of expert microscopy remains a diagnostic priority until a new gold standard is developed.

QBC Microhematocrit Centrifugation Method

Microhematocrit centrifugation with use of the QBC malaria tube (glass capillary tube and closely fitting plastic insert; CBC malaria blood tubes [Becton Dickinson, Tropical Disease Diagnostics, Sparks, MD]) has been used for the detection of blood parasites (20, 21). At the end of centrifugation of 50 to 60 µl of capillary or venous blood (5 min in a QBC centrifuge; 14,387 × *g*), parasites or erythrocytes containing parasites are concentrated into a small, 1- to 2-mm region near the top of the RBC column and are held close to the wall of the tube by the plastic float, thereby making them readily visible by microscopy. Tubes precoated with acridine orange provide a stain which induces fluorescence in the parasites. This method automatically prepares a concentrated smear, which represents the distance between the float and the walls of the tube. Once the tube is placed into the plastic holder (Paraviewer) and immersion oil is applied onto the top of the hematocrit tube (no coverslip is necessary), the tube is examined with a ×40 to ×60 oil immersion objective (there must be a working distance of 0.3 mm or greater) (Fig. 7.10).

Note Although a malaria infection could be detected by this method, *appropriate thick and thin blood films must be examined to accurately identify the species of the organism causing the infection.*

The range of sensitivities for the QBC test has been as low as 55% to >90% compared with microscopy. While most workers think the method is rapid, some think there are some disadvantages such as the high cost of capillary tubes and equipment, problems in species identification and quantification, technical problems, broken capillaries, and the fact that the capillaries cannot be stored for later reference. This may not be the most appropriate approach for a laboratory that receives a small number of specimens for malaria testing, but it could be helpful in other settings. In a recent study of 745 specimens from an area of low endemicity along the Colombian Pacific coast, the agreement be-

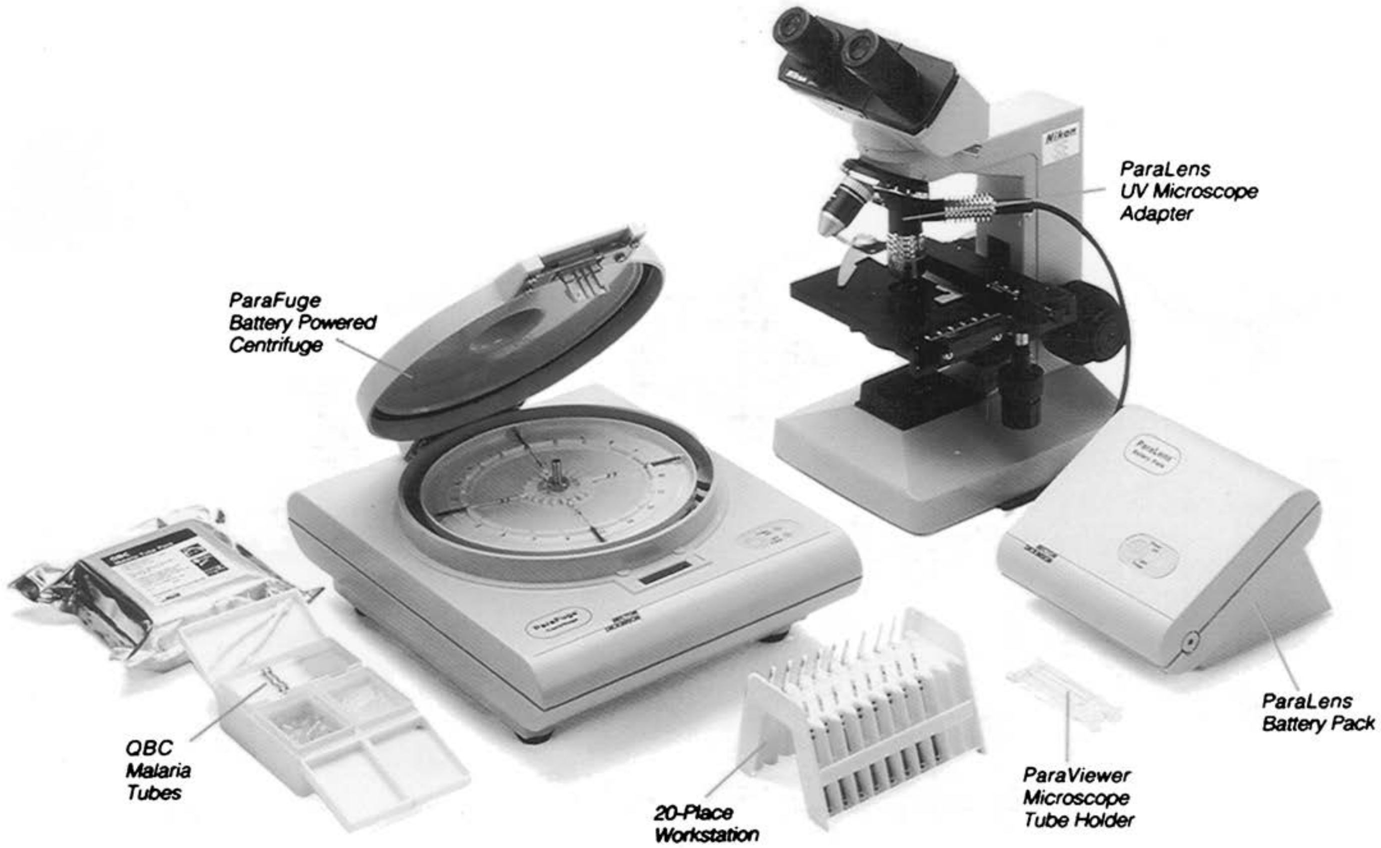


Figure 7.10 The QBC Malaria Test components include the ParaLens UV microscope adapter (blue-violet light module providing fiber-optic illumination with AC outlet or bulb illumination) with rechargeable battery for mobile use (attachable to any conventional microscope), the ParaFuge battery-powered centrifuge, and the ParaViewer microscope tube holder, a specially designed QBC tube viewing block that accepts standard microscope oil. doi:10.1128/9781555819002.ch7.f10

tween the QBC method and thick blood smear was reported to be 99.5% (22). However, another study concluded that in spite of the simplicity of the QBC method, it cannot be considered an acceptable alternative to stained thick blood films in routine laboratory situations (23). An excellent review of this and other methods is provided in reference 24.

This method has been reported to have a high degree of sensitivity in the detection of cases of human filariasis (25). In one study evaluating the technique in the detection of canine *Dirofilaria immitis* as a model for human filariasis, the QBC analysis was more sensitive (55%) than the thick blood smear (39%) and was more efficient (79% versus 72%). However, accurate identification to the species level was impossible using the QBC method.

Diagnosis of tick-borne relapsing fever has also been reported using the QBC system with a high level of sensitivity, more so than the thick blood smear. This method seems to be useful for this purpose and might be considered in the management of fever in travelers returning from tropical regions (26).

ParaSight F Test

A *P. falciparum* antigen detection system, the ParaSight F test (Becton Dickinson), is very effective in field trials (27). This procedure is based on an antigen capture approach and has been incorporated in a dipstick format; the entire test takes approximately 10 min (Fig. 7.11 and 7.12) (27). The overall performance of the ParaSight F test was reviewed in 1995 at a World Health Organization meeting looking at 15 studies with a total of 7,926 assays for the detection of *P. falciparum*. Overall, the sensitivity ranged from 84 to 94%, with specificities ranging from 81 to 99% (28). However, the test detects only *P. falciparum*, and at low parasitemias (100/µl) the sensitivity drops to 11 to 40%. Also, since HRP2 is not present in mature gametocytes, cases where only gametocytes were present could be missed. There is also a possibility that some false-negative results, even at high parasitemias, are due to the lack of the HRP2 gene (29). After successful therapy, many patients continue to have cir-

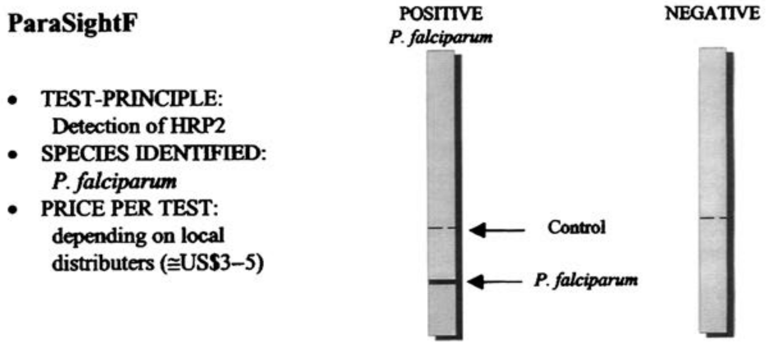
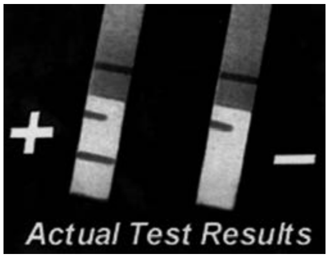

ParaSightF
• TEST-PRINCIPLE: Detection of HRP2
• SPECIES IDENTIFIED: P. falciparum
• PRICE PER TEST: depending on local distributers (≅US$3–5)
POSITIVE
P. falciparum
NEGATIVE
Control
P. falciparum

Figure 7.11 Diagram of the ParaSight F test format. (Adapted from reference 24 with permission.) doi:10.1128/9781555819002.ch7.f11

culating HRP2 antigen for 7 to 14 days after microscopic and clinical cure. Also, as many as 60% of patients positive for rheumatoid factor have a false-positive test (30). The ParaSight F test may be used in situations where no trained microscopists are available or where malaria is strongly suspected and the microscopy results are negative. This kit has proven to be very useful in many areas of the world.

In one systematic review and meta-analysis of controlled studies evaluating the diagnostic accuracy of the ParaSight F test in comparison with light microscopy, data sources included 15,359 subjects (4,119 with *P. falciparum* malaria) in 32 studies reported in 29 publications. Overall, the ParaSight F test demonstrated 90% sensitivity and 94% specificity. Both the sensitivity and specificity were significantly higher in the nonresident population than in the resident population. The posttest probability indicates that in settings of low malaria prevalence a negative test almost absolutely excludes infection, while in settings of high prevalence the same result still gives a substantial chance of infection being present. The authors conclude that the ParaSight F test is a simple and accurate test for the diagnosis of *P. falciparum* infection. The test could be of particular value in the diagnosis of malaria in travelers returning from areas of endemic infection (31).

Figure 7.12 ParaSight F test showing (from left to right) the positive test strip with a reagent control mark above the positive test result and a negative test strip with the reagent control mark. doi:10.1128/9781555819002.ch7.f12

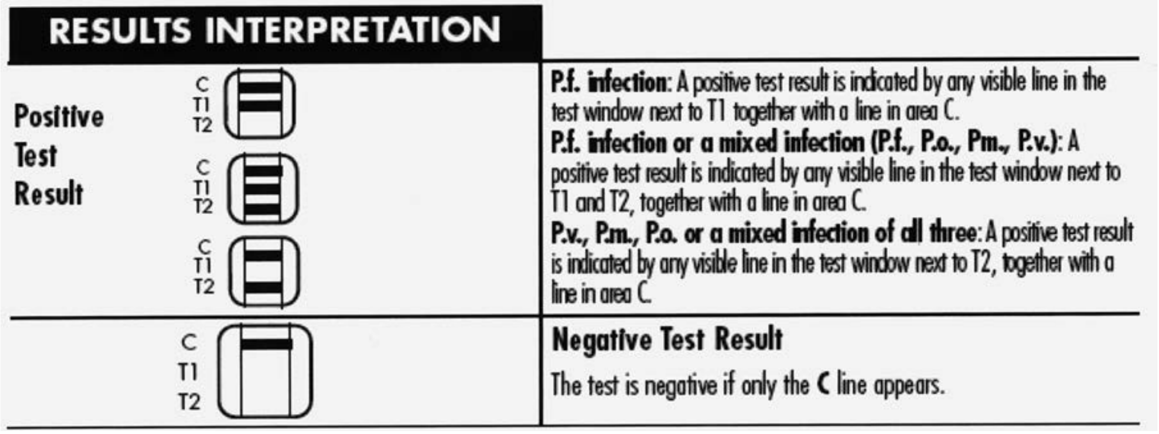


NOW Malaria Test

The ICT Malaria P.f. test (ICT Diagnostics, Brookvale, New South Wales, Australia) was the first malaria rapid diagnostic device designed for convenience of use in a booklet format with the test strip mounted on cardboard. Like other nonmicroscopic malaria rapid tests, ICT Malaria P.f. was an immunochromatographic assay. The original ICT assay detected only *P. falciparum* HRP2. By 1999, AMRAD (French's Forest, New South Wales, Australia) acquired ICT Diagnostics and continued manufacturing the product. At about the same time, the product was enhanced by adding the capability to detect non-*falciparum* malaria. The refined assay was renamed ICT Malaria P.f./P.v. This improvement was achieved by using monoclonal antibodies to capture *Plasmodium* aldolase, in addition to the HRP2 test. *Plasmodium* aldolase is an enzyme of the parasite glycolytic pathway expressed by the blood stages of *P. falciparum* as well as the non-*falciparum* malaria parasites. Monoclonal antibodies against *Plasmodium* aldolase are panspecific in their reaction and have been used in a combined *P. falciparum-P. vivax* immunochromatographic test that targets the panmalarial antigen along with PfHRP2.

In July 2000, AMRAD ceased the production of ICT and sold its ICT division to Binax, Inc. (Portland, ME), where further developmental work was done to refine the test. The new ICT test was released under the name NOW ICT Malaria P.f./P.v. for Whole Blood and is presently called the Binax NOW malaria test for whole blood (Binax, Inc.). The NOW malaria test uses an immunochromatographic format (Fig. 7.13). The test is now available from Alere (Alere, Inc., Waltham, MA) and is FDA approved for use within the United States.

Prior to the development of the improved product (Binax NOW malaria test), in a number of studies testing over 1,300 assays, the overall sensitivity ranged from 80 to 100% (32). Although some of the same issues apply to this test as to the ParaSight F test, the false-positive rate from the presence of rheumatoid factor appears to be less of an issue (33).



P.f. infection: A positive test result is indicated by any visible line in the test window next to T1 together with a line in area C.

P.f. infection or a mixed infection (P.f., P.o., Pm., P.v.): A positive test result is indicated by any visible line in the test window next to T1 and T2, together with a line in area C.

P.v., P.m., P.o. or a mixed infection of all three: A positive test result is indicated by any visible line in the test window next to T2, together with a line in area C.

Negative Test Result

The test is negative if only the **C** line appears.

Figure 7.13 Diagram of the NOW malaria test format. (Adapted from package insert, Binax, Portland, ME.)
doi:10.1128/9781555819002.ch7.f13

In many areas of the world where both *P. falciparum* and *P. vivax* occur, rapid diagnostic tests for malaria must be able to differentiate the two species. It is also interesting that when a mixed-species infection with both *P. falciparum* and *P. vivax* is misdiagnosed as a single-species *P. vivax* infection, treatment for *P. vivax* can lead to a surge in *P. falciparum* parasitemia. The combined *P. falciparum-P. vivax* immunochromatographic test (NOW ICT Malaria Pf/Pv) was used in Indonesia, where both species occur (34). Blinded microscopy was used as the gold standard, with all discordant and 20% of concordant results cross-checked blindly. Of those with a presumptive clinical diagnosis of malaria, only 50% were parasitemic. The NOW ICT Malaria Pf/Pv test was sensitive (95.5%) and specific (89.8%) for the diagnosis of *P. falciparum* malaria, with a positive predictive value of 88.1% and a negative predictive value of 96.2%. Although the specificity and negative predictive value for the diagnosis of *P. vivax* malaria were 94.8 and 98.2%, respectively, the overall sensitivity of 75% and positive predictive value of 50% were lower than desired. With parasitemias of >500/µl, the sensitivity for the diagnosis of *P. vivax* malaria was 96%; with parasitemias of <500/µl, it was only 29%. However, by using this test, undertreatment rates would be reduced from 14.7 to 3.6% with a modest increase in the rate of overtreatment of microscopy-negative patients from 7.1 to 15.4%. Unfortunately, cost remains an obstacle to widespread implementation.

In a study using the improved product (NOW malaria test), the sensitivity for *P. falciparum* was 100% and the specificity was 96%; the sensitivity for *P. vivax* was 89%, and the specificity was 98%. Testing was performed using *P. falciparum*- and *P. vivax*-positive specimens. These results suggest improved performance over NOW ICT predecessors (Fig. 7.13) (35). In another study, the NOW ICT test showed a sensitivity of 94% for the detection of *P. falciparum* malaria (96% for pure *P. falciparum* infection) and 84% for non-*P. falciparum* infections (87% for pure *P. vivax* infections and 62% for pure *P. ovale* and *P. malariae* infections) compared with PCR, with an overall specificity of 99% (36). The Binax NOW ICT may represent a useful adjunct for the diagnosis of *P. falciparum* and *P. vivax* malaria in febrile returned travelers. However, particularly in this patient group and in tests with a negative result, the rapid test should be performed in association with more traditional methods such as examination of thick and thin blood films (37, 38).

Flow Anti-pLDH *Plasmodium* Monoclonal Antibodies

pLDH is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and is present in and released from the parasite-infected erythrocytes. It has been found in all five human malaria species, and different isomers of pLDH for each of the five species exist. With pLDH as the target, a quantitative immunocapture assay, a qualitative immunochromatographic dipstick assay using monoclonal antibodies, an immunodot assay, and a dipstick assay using polyclonal antibodies have been developed.

Flow anti-pLDH *Plasmodium* monoclonal antibodies detect a malaria pLDH antigen by using an antibody incorporated into the dipstick format (39) (Fig. 7.14). The test is positive only when viable parasites are present. Although the test has both a *P. falciparum*-specific and a panspecific antibody against all four species, the panspecific antibody detects only *P. vivax* with any degree of consistency (40, 41). The sensitivity and specificity of the Flow monoclonal antibodies are comparable to those of microscopy in detecting malaria infections at a parasitemia of >100/µl; however, the test failed to identify more than half of the patients with

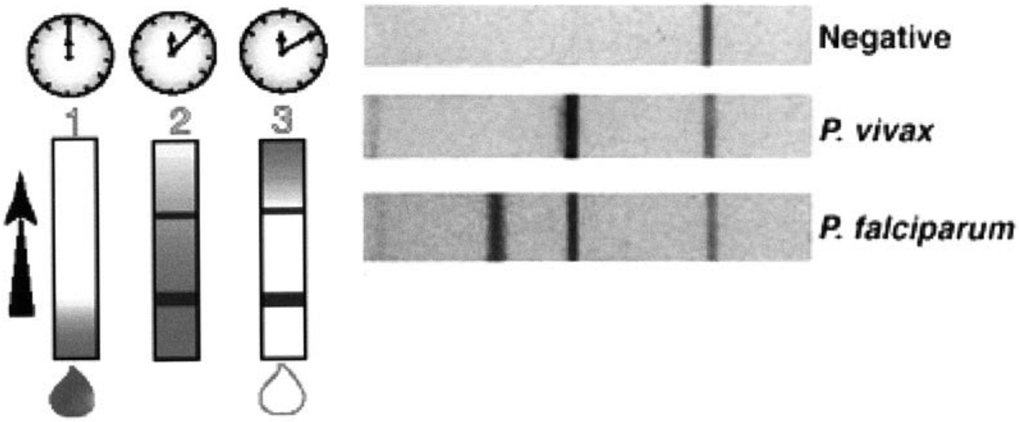


Figure 7.14 Flow anti-pLDH *Plasmodium* monoclonal antibodies rapid malaria test. (Left) Diagram of blood flow on the membrane through the timed test; note the positive control line and positive test line. (Right) Diagram of negative control, a positive *P. vivax* result, and a positive *P. falciparum* result. Note that the line for *P. vivax* is a panspecific antibody against all four species; however, the panspecific antibody has been shown to detect only *P. vivax* with any degree of consistency. (Photographs adapted from Flow's website, with permission.)
doi:10.1128/9781555819002.ch7.f14

a parasitemia of <50/μl. In one study, the sensitivity of the Flow monoclonal antibodies was 97% at a high parasitemia (>100/μl) but fell to 39% at <50/μl (42). Therefore, the test is similar to the HRP2 assays and should not replace conventional microscopy in the diagnosis of malaria infection (43). However, a definite advantage is the ability to confirm a cure, since the test detects only viable organisms. Also, the number of false-positive results due to rheumatoid factor is much smaller than for some of the other tests. In a comparison of results with the ICT Malaria Pf and ParaSight F tests, low monoclonal antibodies correctly identified *P. falciparum* malaria in patient blood samples more often than the other two procedures did. In this study, the Flow monoclonal antibodies exhibited 94% sensitivity and 100% specificity for *P. vivax* and 88% sensitivity and 99% specificity for *P. falciparum* (41). In another study, compared with PCR, the sensitivity was 93% and the specificity was 99.5%. The authors felt that the test had sufficient sensitivity and specificity to detect *P. vivax* under laboratory conditions and could also be useful for malaria diagnosis in the field in Mexico (44). As with the other rapid malaria tests, cost is always an issue; however, Flow monoclonal antibodies provide a simple, rapid, and effective test in the diagnosis of malaria, especially where well-trained microscopists are not available or the work load is too high (45).

Molecular Testing

Although the first PCR methods were developed to identify *P. falciparum*, many can now detect several or all five species. It has been well demonstrated that PCR can detect lower parasitemias than any of the traditional blood film or nontraditional dipstick methods, and it may be preferable to microscopy as the reference standard when evaluating new diagnostic tests (22, 24, 42, 46–48). One advantage of PCR is the ability to confirm false-negative results by microscopy as true positives. Another advantage is the enhanced ability to identify organisms to the species level, particularly in mixed infections, compared with microscopy. However, even PCR may miss some cases as a result of PCR inhibitors, DNA degradation, or genotypic variants. Unfortunately, the time required for PCR presents a problem for the clinical laboratory; this approach does not lend itself to routine use. With the development of automation for PCR, these tests may become much more user-friendly for the routine laboratory; however, currently PCR is reserved for reference centers and special circumstances. As PCR becomes more widely used, it will be important to remember that the mode of collection and storage of blood samples may influence the sensitivity of *Plasmodium* detection. This may be critical in studies of individuals with low parasitemia or mixed infections and in comparison of data from different settings, including field settings.

Other developments include the post-PCR/ligase detection reaction-fluorescent microsphere assay (LDR-FMA) (49). This assay, which uses Luminex FlexMAP microspheres, provides simultaneous, semiquantitative detection of infection by four human malaria parasite species at a sensitivity and specificity equal to those of other PCR-based assays. In blinded studies using *P. falciparum*-infected blood from *in vitro* cultures, the authors identified infected and uninfected samples with 100% concordance. Also, in analyses of *P. falciparum in vitro* cultures and *P. vivax*-infected monkeys, comparisons between parasitemia and LDR-FMA signal intensity showed very strong positive correlations ($r > 0.95$). Application of this multiplex *Plasmodium* LDR-FMA diagnostic assay will increase the speed, accuracy, and reliability of diagnosing human *Plasmodium* infections.

Certainly molecular testing offers many advantages over microscopy, including identification to species levels; this capability is becoming much more important with the shortage of experienced microscopists. A recent multiplex quantitative real-time PCR can rapidly and simultaneously identify all five *Plasmodium* species known to cause malaria in humans (*P. vivax*, *P. ovale*, *P. malariae*, *P. falciparum*, *P. knowlesi*) (50). In another recent study, malaria loop-mediated isothermal amplification (LAMP) used in a remote Ugandan clinic achieved sensitivity similar to that achieved by a single-well nested PCR in a United Kingdom reference laboratory (51).

Automated Blood Cell Analyzers

Although the use of automated blood cell analyzers is not yet clinically relevant for the diagnosis of blood parasites, improvements in the systems may offer future information that will supplement the routine microscopy procedures currently in use (52, 53). Unfortunately, when the parasitemia is light, automation tends to have some of the same problems seen with other alternative procedures. In many cases, the changes seen using analyzers are not specific to malaria infection and could occur in many other diseases (increases in the number of large, unstained cells and thrombocytopenia). The accuracy for malaria diagnosis appears to vary according to the *Plasmodium* species, parasitemia, immunity, and clinical context.

The Cell-Dyn 4000 automated hematology analyzer (Abbott, Chicago, IL) has the ability to detect 91.2% of malaria patients. In one study on day 3 of follow-up post-treatment, the sensitivity was 96.7% that of microscopy. The atypical polarizing events, which indicate the presence of malarial parasites in the analyzer, were highly correlated with the levels of parasitemia in serially diluted samples of the leukocyte-depleted blood; parasites were detected down to the level of 288 ± 17.7/µl). These data suggest that the atypical light depolarization could be influenced by parasitemia and could be used as a screening method for *P. vivax* malaria patients, as well as for therapeutic monitoring (52). Microscopy is still required for species determination and parasite quantitation.

The potential of automated depolarization analysis in detecting malaria infection as part of the routine full blood count performed by the Cell-Dyn 4000 analyzer has been described previously (52). In these cases, abnormal depolarizing patterns are due to the presence of leukocyte-associated malaria hemozoin, a pigment which depolarizes the laser light. Abnormal polarizing events have also been described for samples from three individual patients infected by the nematode *Mansonella perstans*. The observed depolarizing pattern consisted of a normal depolarizing eosinophil population plus an abnormal depolarizing population that showed a close "linear" relationship between "granularity" (90° depolarization) and "lobularity" (90° polarization). This atypical population was smaller than that of normal leukocytes and thus clearly different from the patterns associated with malaria infection. Abnormal depolarization patterns of *M. perstans* clearly do not reflect leukocyte-associated malaria hemozoin. It is possible, however, that the erythrocyte-lysing agent used to facilitate leukocyte analysis by the instrument may have caused microfilaria fragmentation and thus the distinctive straight-line features of the abnormal scatter plots (54).

Diagnosis of Leishmaniasis: Review of Alternatives to Conventional Microscopy

ICT for Detection of Anti-rK-39 Antibodies

In one study, the diagnostic utility of an immunochromatographic test for detection of anti-rK-39 antibodies for the diagnosis of kala azar and post-kala azar dermal leishmaniasis (PKDL) was evaluated (55). Of the 120 samples tested, 57 were positive by ICT; 51 of these were diagnosed as kala azar, and 6 were diagnosed as PKDL. The controls included individuals from areas of endemic (21) and nonendemic infection with malignancies, hemolytic disorders, chronic liver disease, hypersplenism, portal hypertension, metabolic disorders, or sarcoidosis. In addition, 47 sera from patients with confirmed cases of tuberculosis, malaria, typhoid, filariasis, leptospirosis, histoplasmosis, toxoplasmosis, invasive aspergillosis, amebic liver abscess, AIDS, leprosy, cryptococcosis, strongyloidiasis, and cyclosporosis, as well as from patients with collagen vascular diseases and patients with hypergammaglobulinemia, were tested to check the specificity of the test. Of the 51 patients with kala azar, all had fever lasting from <1 month to 1.5 years (median, 4.5 months). All six PKDL patients gave a history of having kala azar in the past, and their slit skin test smears were microscopically positive for Leishman-Donovan bodies. The strip test was positive in all the cases of kala azar and PKDL (estimated sensitivity, 100%), and all control sera were negative by the ICT (specificity, 100%). The rK-39 ICT is a highly sensitive and specific test and may be suitable for a rapid, cost-effective, and reliable field diagnosis of kala azar and PKDL (55).

This test has also been adapted to the use of urine (56) and sputum (57) for the detection of visceral leishmaniasis antibody. Both screening approaches appear to be very promising, with distinct advantages over the invasive use of blood specimens.

Concentration Procedures

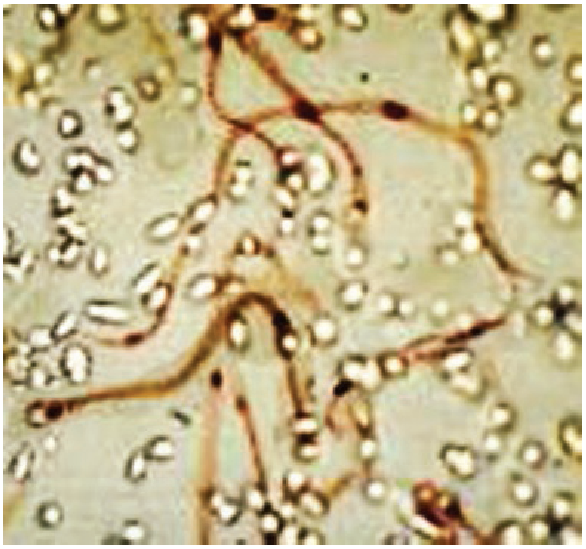
Cytocentrifugation Technique

Cytocentrifugation (cytospin), which uses an apparatus for concentrating cells in suspension on a microscope slide, is commonly used in most histopathology laboratories. The use of a hemolyzing and isotonic saponin solution to lyse RBCs and platelets, which contains formalin as a fixative, has led to an improved technique for the detection of *Plasmodium* spp., *L. donovani*, microfilariae, and WBCs containing malaria pigment. The concentration of the parasites present in the sediment from 100 µl of blood spread on a 6-mm-diameter circle results in good morphology that is well stained using Giemsa, Wright's, or rapid stains. This new method costs very little to perform and offers the possibility of isolating and identifying the main blood-stage parasites in the same sediment. The possible exception would be young trophozoites of *P. falciparum*, which do not concentrate well due to their small size (58).

Knott Concentration Procedure

The Knott concentration procedure is used primarily to detect the presence of microfilariae in the blood, especially when a light infection is suspected (4, 59) (Fig. 7.15). The disadvantage of the procedure is that the microfilariae are killed by the formalin and are therefore not seen as motile organisms.

Figure 7.15 Microfilariae at low power from a Knott concentration, unstained. Note that the sheath is not visible.
doi:10.1128/9781555819002.ch7.f15



1. Place 1 ml of whole or citrated blood into a centrifuge tube containing 10 ml of 2% formalin. Thoroughly mix the tube contents.
2. Centrifuge for 2 min at 500 × *g* or 5 min at 300 × *g*.
3. Decant the supernatant fluid, examine some sediment as a wet mount at low (×100) and high dry (×400) power, prepare thick films from the remaining sediment, and allow the films to air dry.
4. Stain films with Giemsa, Wright's, or rapid stains.

Note Use alcohol-cleaned slides for preparation of the films made from the sediment.

Membrane Filtration Technique

The membrane filtration technique as modified by Desowitz and others has proved highly efficient in demonstrating filarial infections when microfilaremias are of low density. It has also been successfully used in field surveys (3, 4, 60, 61). (See also Fig. 5.9, which shows a membrane filtration system that can also be used for blood filtration; see below.)

1. Draw 1 ml of fresh whole blood or anticoagulated blood into a 15-ml syringe containing 10 ml of distilled water.
2. Gently shake the mixture for 2 to 3 min to ensure that all blood cells are lysed.
3. Place a 25-mm Nuclepore filter (5-µm porosity) over a moist 25-mm filter paper pad. (This method is unsatisfactory for the isolation of *M. perstans* microfilariae because of their small size. A 3-µm-pore-size filter could be used for recovery of this organism. Other filters of similar pore size are not as satisfactory as the Nuclepore filter.) Place the filter in a Swinney filter adapter.
4. Attach the Swinney filter adapter to the syringe containing the lysed blood.
5. With gentle but steady pressure on the piston, push the lysed blood through the filter.
6. Without disturbing the filter, remove the Swinney adapter from the syringe and draw approximately 10 ml of distilled water into the syringe. Replace the adapter, and gently push the water through the filter to wash the debris from the filter.
7. Remove the adapter again, draw the piston of the syringe to about half the length of the barrel, replace the adapter, and push the air in the barrel through the filter to expel excess water.
8. To prepare the filter for staining, remove the adapter, draw the piston about half the length of the barrel, and then draw 3 ml of absolute methanol

into the syringe. Holding the syringe vertically, replace the adapter and push the methanol followed by the air through the filter to fix the microfilariae and expel the excess methanol.

9. To stain, remove the filter from the adapter, place it on a slide, and allow it to air dry thoroughly. Stain with Giemsa stain as for a thick film (with 0.1% Triton X-100) or with Delafield's hematoxylin.
10. To cover the stained filter, dip the slide in toluene before mounting the filter with neutral mounting medium and a coverslip. This will lessen the formation of bubbles in or under the filter.

Gradient Centrifugation Technique

The gradient centrifugation technique is another technique for the concentration of microfilariae (62).

1. Mix 30 ml of 50% Hypaque with 14 ml of distilled water; add 1 part of this mixture to 2.4 parts of 9% Ficoll.
2. Place 4 ml of the Ficoll-Hypaque mixture in a 15-ml plastic centrifuge tube; overlay this mixture with 4 ml of heparinized venous blood.
3. Centrifuge the tube at 400 × *g* for 40 min.
4. Microfilariae will be found in the middle Ficoll-Hypaque layer, which separates the overlying plasma and WBC layers from the underlying RBCs.

Triple-Centrifugation Method for Trypanosomes

The triple-centrifugation procedure may be valuable in demonstrating the presence of trypanosomes in the peripheral blood when the parasitemia is light (4, 9) (Fig. 7.16).

1. Centrifuge anticoagulated blood for 10 min at 300 × *g*.
2. Remove the supernatant fluid, and transfer it to another centrifuge tube.
3. Centrifuge this fluid for 10 min at 500 × *g*.
4. Again, remove the supernatant fluid to another centrifuge tube.
5. Centrifuge this fluid for 10 min at 900 × *g*.
6. Decant the supernatant fluid, and examine the sediment as a wet preparation.
7. The sediment may be used to prepare thin films that can then be stained with any of the blood stains.

Note Remember, you are saving the supernatant fluid in step 2 for subsequent centrifugation; do not accidentally pour this fluid off for disposal. The final sediment after the third centrifugation step is used to prepare stained films for examination.

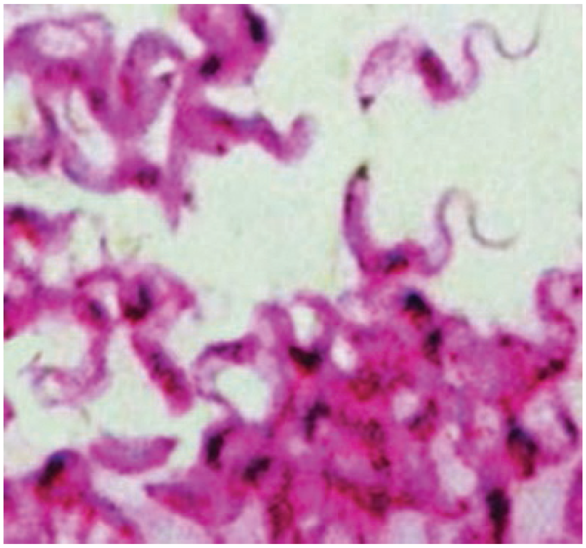


Figure 7.16 African trypanosomes obtained using the triple centrifugation method. doi:10.1128/9781555819002.ch7.f16

Special Stain for Microfilarial Sheath

Delafield's Hematoxylin

Some of the material that is obtained from the concentration procedures can be allowed to dry as thick and thin films and then stained with Delafield's hematoxylin, which demonstrates greater nuclear detail as well as the microfilarial sheath, if present. In addition, fresh thick films of blood containing microfilariae can be stained by this hematoxylin technique (Fig. 7.17 and 7.18) (4, 9).

Preparation of Stain

Dissolve 180 g of aluminum ammonium sulfate in 1 liter of distilled water (saturated solution). Heat until dissolved. The cooled supernatant fluid is saturated aluminum alum.

Hematoxylin Solution

Hematoxylin crystals	4 g
95% ethyl alcohol	25 ml
Ammonium alum (saturated solution)	400 ml

Dissolve the hematoxylin in alcohol, and add it to the alum solution. Expose the solution to sunlight and air for ripening in a clear, cotton-plugged bottle for approximately 1 week; then filter and add the following:

Glycerin	100 ml
95% ethyl alcohol	100 ml

Age for 1 month or longer in sunlight, and then run test smears to determine whether the solution is properly aged. Nuclei should stain blue, and cytoplasm should stain different shades of red.

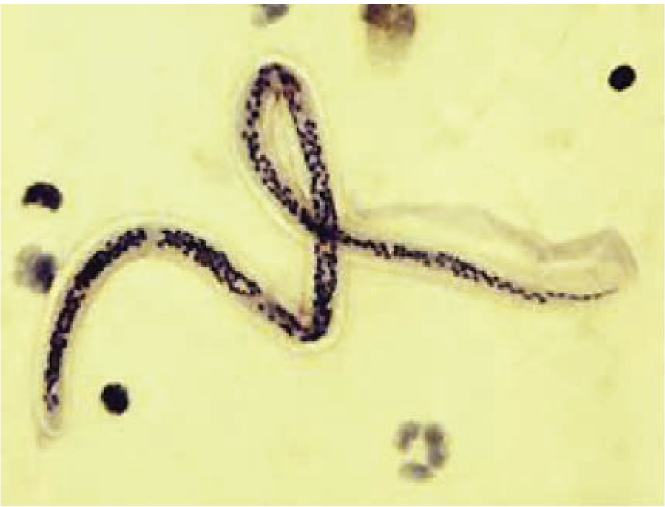
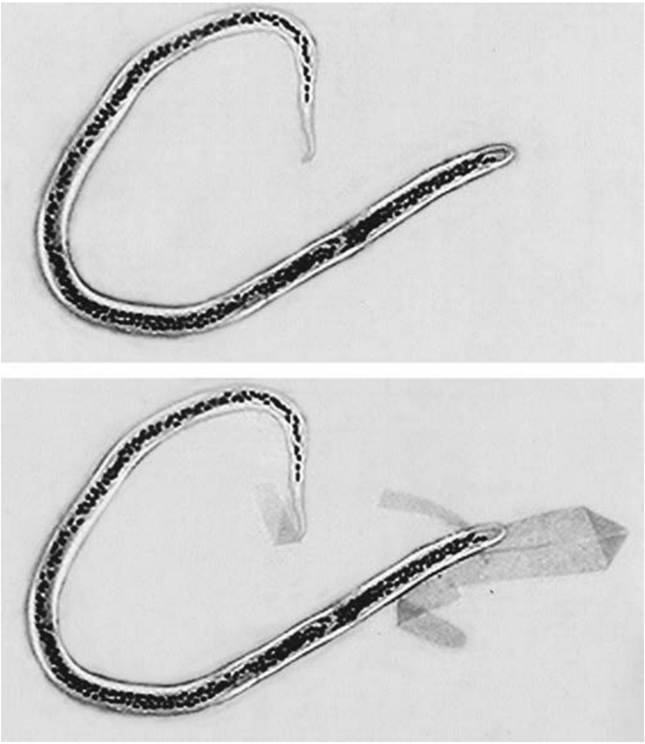


Figure 7.17 *Wuchereria bancrofti* microfilaria on a thick film stained with Delafield's hematoxylin. Note the presence of the sheath. (Image courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch7.f17

Figure 7.18 *Wuchereria bancrofti* microfilaria. (Upper) Microfilaria stained with Giemsa stain. Note that the sheath is not visible. (Lower) Microfilaria stained with Delafield's hematoxylin stain. Note that the sheath is visible. doi:10.1128/9781555819002.ch7.f18



Procedure

1. Prepare thick films from the concentration material or from fresh blood.
2. Allow the films to air dry.
3. Lake the films in 0.85% sodium chloride or distilled water for 15 min (not necessary for films prepared from the Knott sediment). Allow to air dry.
4. Fix all films in absolute methanol for 5 min. Allow to air dry.
5. Stain in undiluted Delafield's hematoxylin for 10 to 15 min.
6. Wash off excess stain in tap water.
7. Intensify the blue color by placing the films into tap water containing several drops of ammonia (NH_4OH) for several minutes.
8. Rinse again in running tap water for 5 min.
9. Air dry.
10. Mount in Permount or some other mounting medium; use a no. 1 coverglass.

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8

Parasite Recovery: Culture Methods, Animal Inoculation, and Xenodiagnosis

Culture methods
Intestinal protozoa
Pathogenic free-living amebae
Blastocystis spp. (*Blastocystis hominis*)
Pathogenic flagellates
Flagellates of blood and tissue
Toxoplasma gondii
Plasmodium and *Babesia* spp.
Cryptosporidium spp.
Microsporidia
Animal inoculation
Leishmania spp.
Trypanosoma spp.
Toxoplasma gondii
Xenodiagnosis

Culture Methods

Very few clinical laboratories offer specific culture techniques for parasites. The methods for *in vitro* culture are often complex, while quality control is difficult and not really feasible for the routine diagnostic laboratory. In certain institutions, some techniques may be available, particularly when consultative services are provided (reference laboratory situation) and for research purposes. However, most laboratories do not offer these techniques.

Few parasites can be routinely cultured; however, methods are available for *Entamoeba histolytica*, *Naegleria fowleri*, *Acanthamoeba* spp., *Trichomonas vaginalis*, *Dientamoeba fragilis*, *Toxoplasma gondii*, *Blastocystis* spp., *Trypanosoma cruzi*, and the leishmanias. Often, when specimens are cultured for potential pathogens, nonpathogenic protozoa could also be recovered. These procedures are usually available only after consultation with the laboratory and on special request. For those who may be interested in trying these techniques, the several different media presented below are representative of those available. More extensive options can be found in the literature (1–13).

Cultures of parasites grown in association with an unknown microbiota are referred to as xenic cultures. A good example of this type of culture would be stool specimens cultured for *E. histolytica*. If the parasites are grown with a single known bacterium, the culture is referred to as monoxenic. An example of this type of culture would be clinical specimens (corneal biopsy specimens) cultured with *Escherichia coli* as a means of recovering species of *Acanthamoeba* and *Naegleria*. If parasites are grown as pure culture without any bacterial associate, the culture is referred to as axenic. An example of this type of culture would be the use of media for the isolation of *Leishmania* spp. or *Trypanosoma cruzi*. All three types of cultures are discussed in this chapter.

Pancreatic digests of casein are major ingredients of media used in the axenic cultivation of lumen-dwelling parasitic protozoa. Unfortunately, the digest used almost exclusively in the development of these media has not been available since the early 1980s. Many digest products have been tried in the interim with marginal results in supporting the growth of *E. histolytica*. Diamond et al. (14) have developed a casein-free medium, YI-S, consisting of a nutrient broth, vitamin mixture, and serum. This may serve as a replacement for TYI-S-33, widely used for the axenic culture of *E. histolytica* and other intestinal protozoa.

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Intestinal Protozoa

Specimens include stool material, mucus, or a combination of the two. The clinical specimen(s) should be no more than 24 h old; a maximum of 2 to 3 h is recommended.

Balamuth's Aqueous Egg Yolk Infusion Medium for Amebae

Balamuth's aqueous egg yolk infusion medium is used to detect the presence of amebae. The specific solutions required are phosphate buffer and whole-liver concentrate solution.

Balamuth's Aqueous Egg Yolk Infusion Medium

Phosphate buffer

(A) Potassium phosphate, tribasic (K_3PO_4)	212.27 g
Distilled water	1,000 ml
(B) Potassium phosphate, monobasic (KH_2PO_4)	136.092 g
Distilled water	1,000 ml

Mix the solution in the ratio of 3 parts tribasic (A) to 2 parts monobasic (B) to make 1 M phosphate buffer stock. Dilute the stock buffer to 0.067 M before use (add 492 ml of distilled H_2O to 1 liter of 1 M phosphate buffer).

Whole-liver concentrate solution

Liver concentrate powder (Wilson or Lilly)	5 g
Distilled water	100 ml

Suspend the powder in cold water, and autoclave. Filter through a Büchner funnel to remove sediment, dispense in 10-ml quantities, and reautoclave.

Preparation of Complete Medium (15)

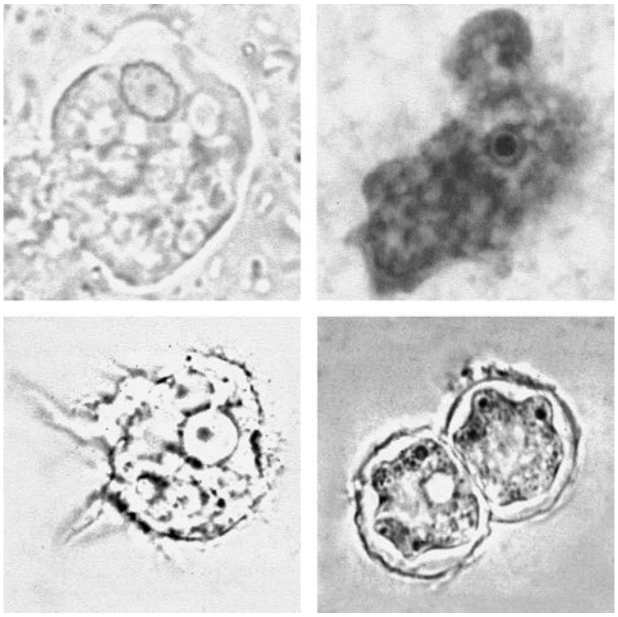
1. Using a blender, blend 12 fresh hard-boiled egg yolks with 375 ml of 0.8% sodium chloride.
2. Autoclave at 7 lb/in.^{2} pressure for 10 min, repressurize slowly, and stir.
3. Autoclave again for 45 min at 7 lb/in.^{2} pressure.
4. Allow to cool slightly, and add distilled water to replace evaporation loss. Transfer the material to a muslin bag, and express the liquid portion, saving all the fluid. Return the volume to 375 ml with 0.8% sodium chloride.
5. Autoclave for 20 min at 121°C; cool to 5°C. Do not agitate the fluid at this point or during filtration.
6. Decant the fluid carefully through gauze into a Büchner funnel with Whatman no. 3 filter paper. Filter papers can be replaced as necessary.
7. Measure the filtrate, add an equal volume of 0.067 M phosphate buffer, and autoclave for 20 min at 121°C. After cooling, add stock liver concentrate (1 part stock liver to 9 parts medium).
8. Material can then be decanted into sterile flasks, which are stored until the medium is dispensed.

Procedure

Tubes should contain 6 to 8 ml of fluid and should be incubated for 4 days at 37°C as a sterility check. Before inoculation, a loopful of sterile rice powder or starch (can be autoclaved in a screw-cap tube) is added to each tube. To each tube, add stool material, mucus, or a combination of the two (about the size of a small pea), break it up thoroughly in the medium, and incubate at 37°C. The cultures should be checked at 2, 3, and 4 days by examining 0.1 ml of sediment under the microscope (low-intensity light) for characteristic motility. Although the initial culture may appear to be negative, subcultures may reveal organisms (Fig. 8.1).

According to Dolkart and Halpern (16), the addition of gastric mucin to the egg component is reported to improve the performance of Balamuth's medium.

Figure 8.1 Protozoa from culture systems. (Upper left) *Entamoeba histolytica/E. dispar* trophozoite from liquid medium containing rice starch (note that there are no definitive erythrocytes within the cytoplasm, so that it is not possible to differentiate the true pathogen, *E. histolytica*, from the nonpathogen, *E. dispar*). (Upper right) *Naegleria fowleri* trophozoite from nonnutrient agar culture with bacterial overlay (note that this trophozoite has been stained). (Lower left) *Acanthamoeba* spp. trophozoite from nonnutrient agar culture with bacterial overlay (note the spiky acanthapodia). (Lower right) *Acanthamoeba* spp. cysts from nonnutrient agar culture with bacterial overlay (note the double hexagonal wall appearance. doi:10.1128/9781555819002.ch8.f1



The addition of rice flour (prerinsed with distilled water) to Balamuth's medium is reported to support abundant growth of *Balantidium coli*.

Boeck and Drbohlav's Locke-Egg-Serum (LES) Medium for Amebae

Boeck and Drbohlav's LES medium is another culture medium used to diagnose the presence of amebae.

Boeck and Drbohlav's LES Medium

Locke's solution

NaCl	9.0 g
$CaCl_2$	0.2 g
KCl	0.4 g
$NaHCO_3$	0.2 g
Glucose	2.5 g
Distilled water	1,000 ml

Autoclave before storage.

Preparation of Complete Medium

1. Wash four eggs, wipe the shells with 70% alcohol, and break the eggs into a sterile flask containing glass beads.
2. Add 50 ml of Locke's solution, and shake until homogenous.
3. Dispense the medium so that a slant of 1 to 1.5 in. (1 in. = 2.54 cm) is produced in the bottom of the tube. (Tube size is not critical.)
4. Plug the tubes, and place them in a slant position in an inspissator at 70°C until the slant solidifies. Inspissator conditions may be achieved in the autoclave by leaving the door ajar (nonpressurized system).
5. Autoclave the tubes at 121°C for 20 min. Discard any damaged slants.
6. Prepare a mixture of 8 parts sterile Locke's solution to 1 part sterile inactivated human serum. Sterilize the mixture by filtration, and incubate at 37°C for 24 to 48 h as a sterility check before use. Cover the slants to a depth of <1 cm with the sterile solution, and inoculate in the same manner as for Balamuth's medium. LES medium should have a loopful of sterile rice powder added before inoculation.

TY1-S-33 Medium for *Entamoeba histolytica* (17)

TYI Broth

Biosate (BBL) Peptone	30.0 g
Dextrose (do not use glucose)	10.0 g
NaCl	2.0 g
L-Cysteine HCl (Sigma)	1.0 g
Ascorbic acid (J. T. Baker)	0.2 g
K_2HPO_4	1.0 g
KH_2PO_4	0.6 g
Ferric ammonium citrate (Mallinckrodt)	22.8 mg
Distilled water up to	870 ml

Note Biosate may be replaced with 20 g of Trypticase (BBL) and 10 g of yeast extract (BBL). Some lots of Biosate, Trypticase, yeast extract, or serum may inhibit growth.

1. Adjust pH to 6.8 with 1 N NaOH, and filter through Whatman no. 1 paper. Autoclave at 121°C for 15 min.
2. Cool, and add:

Heat-inactivated bovine serum (shelf life, 6 months at 4°C)	100 ml
Special 107 Vitamin Mix (below)	30 ml

3. Aseptically dispense 13 ml of complete medium into 16- by 125-mm screw-cap tubes. To prepare Special 107 Vitamin Mix, aseptically combine the following:

Solution 1 (see below)	0.4 ml
Solution 2 (see below)	1.2 ml
Solution 3 (see below)	0.4 ml
Sterile distilled water	18.0 ml
Diamond's TPS-1, vitamin solution, 40× (NABI) diluted to 1×	100.0 ml

Total volume of complete Special 107 Vitamin Mix is 120 ml.

Solution 1 (Filter Sterilized)

D,L-6,8-Thioctic acid, oxidized (Sigma)	100 mg
Absolute ethanol	100 ml

Solution 2 (Filter Sterilized)

Vitamin B_{12} (Sigma)	40 mg
Distilled water	100 ml

Solution 3 (Filter Sterilized)

Tween 80 (Sigma)	50 g
Absolute ethanol	100 ml

Note The shelf life of solutions 1, 2, and 3 is 22 months.

TYI-S-33 (Keister's Modification) for *Giardia lamblia*

For TYI-S-33 (Keister's modification [2]), prepare TYI broth exactly as for *E. histolytica* with the following changes.

(i) Increase the amount of L-cysteine hydrochloride to 2.0 g/liter.
(ii) Add 500 mg of dehydrated bovine bile per liter.
(iii) Adjust the pH to 7.0 to 7.1.
(iv) Sterilize by filtration through a 0.22-µm-pore-size filter. Do not autoclave! The complete medium is made by addition of bovine serum to 10%.

TYSGM-9 Medium for *Entamoeba histolytica*

Nutrient Broth

Potassium phosphate, dibasic (K_2HPO_4)	2.8 g
Potassium phosphate, monobasic (KH_2PO_4)	0.4 g
NaCl	7.5 g
Casein digest peptone (BBL, product no. 97023)	2.0 g
Yeast extract (BBL)	1.0 g
Glass-distilled water	970.0 ml

The nutrient broth may be stored for several months at –2°C.

5% Tween 80 Solution

1. Vigorously stir, with a magnetic stirrer, 95 ml of glass-distilled water in a bottle.
2. Add 5 g of Tween 80 (very thick solution; must be weighed), and keep stirring for a few minutes.
3. Filter sterilize through a 0.22-µm-pore-size membrane.
4. Aseptically dispense into a number of sterile screw-cap test tubes, 10 ml per tube.
5. Label as 5% Tween 80 solution, with the preparation date and an expiration date of no longer than 1 month. Store at 4°C.

Phosphate-Buffered Saline (PBS no. 8), pH 7.2

NaCl	9.5 g
K_2HPO_4	3.7 g
KH_2PO_4	1.1 g
Glass-distilled water to	1,000.0 ml

1. Dissolve the salts in the distilled water with a magnetic stirrer.
2. Autoclave for 15 min at 121°C.
3. When cool, label as PBS no. 8 with the preparation date and an expiration date of 3 months.

Rice Starch

For best results use rice starch obtained from BDH, Ltd. (Merck, Ltd.) or Gailard Schlesinger, Inc.

1. Dispense 500 mg of rice starch into each of several 16- by 125-mm screw-cap tubes; do not tighten the caps.
2. Place the tubes horizontally in a dry-heat sterilizer or an oven. Make sure that the rice starch is uniformly distributed loosely over the undersurface of the tubes.
3. Heat the tubes for 2.5 h at 150°C.
4. When cool, tighten caps and label as rice starch with the date of preparation and an expiration date of 3 months.

Rice Starch Suspension

1. Add 9.5 ml of sterile PBS no. 8 to each tube of rice starch.
2. Shake vigorously or use a vortex machine to uniformly suspend the rice starch at the time of use.

Stock Antibiotic Solution

1. Using a 6-ml syringe and 20-gauge needle, add 5 ml of sterile distilled water to a vial of penicillin G sodium (10^6 U).
2. Using a 6-ml syringe and 20-gauge needle, add 5 ml of sterile distilled water to a vial of streptomycin sulfate (10^6 µg/ml).
3. Shake gently, and let stand for 30 min to dissolve the antibiotics completely in the distilled water.
4. Mix the two antibiotics in a graduated flask or cylinder, and bring the volume to 125 ml with distilled water. The stock concentration of antibiotics is 8,000 U of penicillin/ml and 8,000 µg of streptomycin/ml.
5. Filter sterilize the antibiotic solution through a 0.22-µm-pore-size membrane filter, dispense the filtrate into a number of sterile screw-cap vials or sterile cryovials (1 ml per vial), and label as stock antibiotic solution with the preparation date and an expiration date of 6 months. Store at 120°C in a cryovial box.

Buffered Methylene Blue Solution

Solution A, 0.2 M acetic acid

Glacial acetic acid	11.55 ml
Distilled water	988.50 ml

Add the acetic acid to the water, mix, and store in a glass-stoppered bottle. Label with the date of preparation and an expiration date of 1 year.

Solution B, 0.2 M sodium acetate

Sodium acetate ($CH_3COONa \cdot 3H_2O$)	16.4 g
Distilled water to	1,000.0 ml

Dissolve the sodium acetate in 400 ml of distilled water in a volumetric flask, bring the volume to the 1,000-ml mark, mix well, and store in a glass-stoppered bottle. Label with the date of preparation and an expiration date of 1 year.

Acetate buffer, pH 3.6

Solution A	46.3 ml
Solution B	3.7 ml
Distilled water to	100.0 ml

Mix solutions A and B in a volumetric flask, and bring the volume to 100.0 ml with distilled water. The pH should be 3.6. Store in a glass-stoppered bottle. Label with the date of preparation and an expiration date of 1 year.

Methylene blue stain

Methylene blue dye	60.0 mg
Acetate buffer (from previous step)	100.0 ml

Dissolve the dye in the buffer, and store in a glass-stoppered bottle. Label with the date of preparation and an expiration date of 1 year.

Complete Medium (TYSGM-9 Medium)

1. Place 200 mg of gastric mucin (U.S. Biochemical Corp., catalog no. 16025) in a 125-ml screw-cap bottle or Erlenmeyer flask.
2. Add 97 ml of nutrient broth; using a magnetic stirrer, stir vigorously for at least 1 h or until the medium becomes clear.
3. Autoclave for 15 min at 121°C; cool to room temperature.
4. Add aseptically, in a biological safety cabinet, 5.0 ml of heat-inactivated bovine serum.
5. Add 0.1 ml of the 5% Tween 80 solution.
6. Dispense aseptically, in a biological safety cabinet, into a number of sterile 16- by 125-mm screw-cap tubes, 8 ml per tube.
7. Add 0.25 ml of rice starch solution after vigorously shaking the tube.
8. Store the tubes at 4°C for not more than 1 month.
9. The final pH of the medium should be 7.2.

Robinson's Culture

Robinson's medium is a complex medium that has nevertheless found widespread use for the isolation of enteric amebae. To prepare Robinson's medium, prepare the six following stock solutions (2).

Solution 1 (0.5% Erythromycin) (Filter Sterilized)

Prepare 0.5% erythromycin in distilled water and filter sterilize. Refrigerate.

Solution 2 (20% Bacto Peptone) (Autoclave)

Prepare 20% Bacto Peptone in distilled water. Autoclave and refrigerate.

Solution 3 (10× Phthalate Solution, Stock) (Autoclave)

Potassium hydrogen phthalate	102 g
40% sodium hydroxide	50 ml

Bring to 1 liter at pH 6.3. Autoclave for 15 min at 121°C under a pressure of 15 lb/in.^{2}. Store at room temperature. Dilute 1:10 with sterile water before use.

A stock solution of phthalate-Bacto Peptone can be made by adding 1.25 ml of 20% Bacto Peptone per 100 ml of 1× phthalate solution. Store refrigerated.

Solution 4 (10× R Medium Stock) (Autoclave)

NaCl	25.0 g
Citric acid	10.0 g
Potassium phosphate, monobasic (KH_2PO_4)	25.0 g
Ammonium sulfate	5.0 g
Magnesium sulfate · $7H_2O$	0.25 g
85% lactic acid solution	20 ml

Bring to 500 ml. Dilute stock 1:10, adjusting pH to 7.0. Autoclave for 15 min at 121°C under a pressure of 15 lb/in.^{2} in 20-ml amounts.

Solution 5 (BR Medium)

To prepare BR medium, inoculate 1× R medium with a standard *Escherichia coli* strain such as O111. Incubate at 37°C for 48 h, and store at room temperature (good for several months).

Solution 6 (BRS Medium)

To prepare BRS medium, add an equal volume of heat-inactivated bovine serum to BR medium and incubate at 37°C for 24 h. Store at room temperature (good for several months).

1. To prepare agar slants, many people use screw-cap glass bijou bottles (total volume, 7 ml), but standard culture tubes also work well.
2. Autoclave a solution of 1.5% Noble agar in 0.7% NaCl-distilled water for 15 min at 121°C under a pressure of 15 lb/in.^{2}.
3. Dispense in 5-ml (tube) or 3-ml (bottle) amounts, reautoclave, and slant until cool and set. For slants in tubes, use an angle that produces a 12- to 15-mm (ca. 0.5-in.) butt.
4. When cool, tighten lids and store at room temperature or refrigerated.
5. To one tube or bottle, add the following: 3 ml of 1× phthalate-Bacto Peptone, 1 ml of BRS medium, and 50 µl of erythromycin. This must be done on the same day as the inoculation. Note that although erythromycin is added to Robinson's medium at every subculture, this does not lead to a monoxenic culture as occasionally stated. Additional antibiotic treatment would be needed for this to be the case.

Xenic Culture

When initiating xenic cultures, the stool samples should be inoculated into at least two tubes, one with and the other without antibiotics. In some cases, some component of the natural bacterial flora may be helpful or even necessary for the amebae to become established.

1. Warm several tubes of TYSGM-9 medium in the incubator (35°C for 1 to 2 h).
2. Add 0.1 ml of stock antibiotics to each tube of medium. The final concentration of antibiotics is

100 U of penicillin per ml and 100 µg of streptomycin per ml.

3. After vortexing or vigorously shaking the tube, use a Pasteur pipette to add 3 drops of the starch suspension to each tube of the medium.
4. Place a pea-sized portion of fresh stool sample into the bottom of the tube, and break up the stool gently with the pipette.
5. Tightly cap the tubes, and incubate at a 45 to 50° angle at 35°C for 48 h.
6. Examine the tubes with an inverted microscope and the 10× objective for the presence of amebae. Amebae, if present, are usually seen attached to the underside of the tubes, interspersed with the fecal material and rice starch. Sometimes it is necessary to gently invert the tubes in order to disperse the stool material and rice starch to uncover the amebae. If you do not have an inverted microscope, proceed to step 13.
7. If amebae are not seen, stand the tubes upright for about 30 min at 35°C.
8. With a Pasteur pipette, remove from the bottom of each tube the entire sediment and inoculate the sediment into fresh tubes containing rice starch and antibiotics.
9. Incubate as above for another 48 h.
10. Examine the tubes as before and discard the tubes if amebae are still not seen. Report patient results as negative.

Note These patient results should not be reported unless the quality control organisms and cultures are growing, thus indicating the culture system is performing according to expected results.

11. If amebae are present in small numbers, chill the tube in ice-water for 5 min and centrifuge the tube for 5 min at 250 × *g*. Aspirate and discard the supernatant, and inoculate the sediment into a fresh tube as before.
12. If amebae are present in large numbers, let the tube stand upright for 30 min and remove about 0.2 ml of sediment from the bottom. Inoculate the sediment into fresh tubes as before.
13. If you do not have an inverted microscope, stand the tubes upright for about 30 min at 35°C. With a sterile pipette, remove about 0.5 ml of sediment from the bottom of the tube and place a couple of drops onto each of two slides. Add 2 drops of methylene blue solution to one of the slides. Cover both with coverslips, and examine the slides under the microscope for amebae. Amebae may appear rounded or with pseudopodial extrusions. The nuclei may be clearly seen in the methylene blue preparation. Proceed to steps 7 through 12.

Axenic Culture

Axenic culture is used for research when strains of organisms are necessary for work requiring a culture system free from bacterial contaminants. If the axenic culture tubes become contaminated, 1,000 U of penicillin per ml and 1,000 µg of streptomycin or 50 µg of gentamicin per ml can be added to each tube. If, however, the contaminant happens to be *Pseudomonas* spp., it is probably better to discard the tube and use an uncontaminated tube for subculture purposes (3, 15).

1. Remove tubes containing TYI-S-33 medium from 4°C, and incubate at 35°C for 1 to 2 h.
2. With an inverted microscope, examine stock culture tubes of *E. histolytica* for any signs of bacterial contamination (no longer acceptable for use). Select one or several tubes showing good growth of amebae. Since the tubes are incubated in a slanted position, usually at an angle of 5 to 10°, a thick button of amebae will be seen at the bottom of the tube. Gently invert the tubes once or twice to disperse the amebae uniformly, and examine the tubes again. A majority of the amebae should be attached to the tube walls and show pseudopodial motility. If you do not have an inverted microscope, examine organisms from the bottom of the tube (as a wet smear). If you can see pseudopodial motility, proceed to step 3.
3. Immerse the tubes in a bucket of ice-cold water for about 5 to 10 min to dislodge the amebae from the tube walls. Invert the tubes several times to distribute the amebae.
4. Remove, with a Pasteur pipette, about 1.0 to 1.5 ml of culture medium; inoculate 0.5 to 1 ml into a fresh tube. Inoculate the rest of the fluid into nutrient agar, brain heart infusion, and thioglycolate broth for routine monitoring of bacterial contamination. Inoculate several tubes this way, and incubate the cultures slanted at 5 to 10° at 35°C as before.
5. If amebic growth is not good but some amebae are attached to the tube walls, remove about 10 ml of medium from the bottom with a serologic pipette and add 10 ml of fresh medium.
6. If amebic growth is not good and only few amebae are present along with a lot of debris, centrifuge the tube at 250 × *g* for 10 min, aspirate the supernatant fluid, and transfer the sediment to a fresh tube and incubate as before.

Quality Control for Intestinal Protozoan Cultures

The following control strains should be available when using these cultures for clinical specimens (3): ATCC 30925 (*Entamoeba histolytica* HU-1:CDC), ATCC 30015

(*Entamoeba histolytica* HK-9), and ATCC 30042 (*Entamoeba histolytica*-like, Laredo strain, culture at 25°C) (17).

1. Check all reagents and media (Balamuth's aqueous egg yolk infusion medium, Boeck and Drbohlav's LES medium, PBS solution no. 8, rice starch suspension, Tween 80 solution, and TYSGM-9 and TYI-S-33 media) each time they are used or periodically (once a week). The media and all solutions should be free of any signs of precipitation and bacterial and/or fungal contamination.
2. Maintain stock cultures of *E. histolytica* at 35°C (ATCC 30925 [strain HU-1:CDC] and ATCC 30015 [strain HK-9]). Maintain *E. histolytica*-like Laredo strain (ATCC 30042) at 25°C.
   A. Transfer stock culture (ATCC 30925) every other day with TYSGM-9 medium.
   B. Transfer stock culture (ATCC 30015) once every 3 days with TYI-S-33 medium.
   C. Transfer stock culture (ATCC 30042) once a month with TYSGM-9 medium.
   D. *E. histolytica* trophozoites measure 10 to 60 μm and demonstrate directional motility by extruding hyaline, finger-like pseudopodia from the cytoplasm. Cysts are not usually found in cultures.
   E. Trophozoites are uninucleate and characterized by finely granular, uniform, evenly distributed peripheral chromatin. The nucleolus is small and usually centrally located but may be eccentric.
3. Depending on its use, the microscope(s) may need to be calibrated (within the last 12 months), and the original optics used for the calibration should be in place on the microscope(s). The calibration factors for all objectives should be posted on the microscope for easy access.

Note If the tubes containing fecal material are positive for amebae after 48 h of incubation, confirm the identification with the permanent stained smear. If the tubes do not show any amebae, subculture the contents of the tubes as described above and incubate for an additional 48 h. If the tubes are still negative for amebae, report the specimen as negative and discard the tubes. Even when the culture system is within quality control guidelines, a negative culture is still not definitive in ruling out the presence of *E. histolytica*. Xenic cultures of *E. histolytica* serve only as a supplemental procedure and never replace the primary diagnosis by microscopic examination of concentration sediments and permanent stained smears. Axenic culture is used to maintain quality control strains and for research purposes.

Pathogenic Free-Living Amebae

Specimens would include cerebrospinal fluid (CSF), biopsy tissue, and autopsy tissue of the brain; for *Acanthamoeba* spp., corneal scrapings or biopsy material, contact lenses and contact lens paraphernalia such as lens cases and solutions, skin abscess material, ear discharge, or feces can also be used. All clinical specimens should be processed within 24 h; 2 to 3 h is recommended. However, eye-related specimens can be shipped by mail with apparently few problems (18–21). The procedure for growing *Naegleria* and *Acanthamoeba* from clinical specimens involves the use of a nonnutrient agar spread with *E. coli* or some other nonmucoid bacteria. Amebas begin feeding on bacteria and soon grow to cover the agar surface in 1 to 2 days at 37°C. The presence of the protozoa can be confirmed by examining the agar surface using an inverted microscope or with a conventional microscope by inverting the plate on the stage and focusing through the agar with a 10× objective (Fig. 8.1).

Diagnostic methods include direct microscopy of wet mounts of CSF or stained smears of CSF sediment, light or electron microscopy of tissues, *in vitro* cultivation of *Acanthamoeba*, and histologic assessment of frozen or paraffin-embedded sections of brain or cutaneous lesion biopsy material. Immunocytochemistry, chemifluorescent-dye staining, PCR, and analysis of DNA sequence variation also have been used for laboratory diagnosis. Several approaches to specimen handling and diagnostic methods can be seen in Table 8.1.

Environmental Issues

There continue to be ongoing discussions and publications regarding the association of free-living amebae and their intracellular bacterial flora, particularly within the context of environmental transmission of infection, contamination of equipment, and overall environmental concerns. The recognition that *Acanthamoeba* spp. can sequester a variety of bacteria with known potential for causing human disease suggests that these amebae serve as reservoirs for bacterial pathogens. The increase in the reported incidence of *Acanthamoeba* infections may be due to greater recognition of the disease potential of these amebae. Also, a number of factors may account for an increased incidence of infection, such as a large number of HIV-infected individuals and more patients undergoing chemotherapy or immunosuppressive therapy for organ transplantation (22).

Acanthamoeba Medium

For the isolation of *Naegleria* or *Acanthamoeba* spp. from tissues or soil samples, the following procedure is recommended.

TABLE 8.1 Approaches to isolation of free-living amebae[a]

Approach 1	Approach 2	Approach 3	Approach 4
Tissue sample (central nervous system tissue, corneal scrapings, skin scrapings or biopsy specimen)	Tissue sample (central nervous system tissue, corneal scrapings, skin scrapings or biopsy specimen)	CSF	Environmental sample (soil, water)
Antibiotics (Pen-Strep, gentamicin, amphotericin B)	Nonnutrient agar (plus *Escherichia coli*)	Wet mounts, stains	Nonnutrient agar (plus *Escherichia coli*)
Tissue culture (monkey kidney, rat glioma, human lung fibroblasts)	Wet mounts, stains; enflagellation (identification of *Naegleria*)	Nonnutrient agar; wet mounts, stains; enflagellation (identification of *Naegleria*)	Wet mounts, stains; enflagellation (identification of *Naegleria*)

[a] The decision to examine a specimen as a wet mount and/or permanent stained smears will depend on the amount of specimen received. If the amount is quite small, direct inoculation of nonnutrient agar seeded with bacteria is recommended. Once organisms have been isolated, they can be frozen. (Adapted from reference 6.) It is also important to remember that although different incubation temperatures are recommended for particular organisms, incubation at 37°C is recommended if the specimen is quite small. Do not divide a very small specimen just to use variable incubation temperatures.

Acanthamoeba Medium

Page's saline (10×)

NaCl	60 mg
$MgSO_4 \cdot 7H_2O$	2 mg
$CaCl_2 \cdot 2H_2O$	2 mg
Na_2HPO_4	71 mg
KH_2PO_4	68 mg
Distilled water	500 ml

1. Autoclave at 121°C for 15 min.
2. Store refrigerated in a glass bottle for up to 6 months.

Nonnutrient agar

Page's saline (10×)	100 ml
Difco agar	15 g
Double-distilled water	900.0 ml

1. Dissolve agar in Page's saline and distilled water with gentle heating; stir or swirl.
2. Aliquot 20 ml into screw-cap tubes (20 by 150 mm).
3. Autoclave at 15 lb/in.^{2} for 15 min; label deeps with 12-month expiration date, and store in the refrigerator.
4. Melt agar deeps, and pour into petri dishes as needed. Plates may be stored in the refrigerator for up to 3 months.

Monoxenic culture

1. Remove the nonnutrient agar plates from the refrigerator, and place them in a 37°C incubator for 30 min.
2. Add 0.5 ml of ameba saline to a slant bacterial culture of *E. coli* or *Enterobacter aerogenes*. Gently scrape the surface of the slant (do not break the agar surface). Suspend the bacteria uniformly by gently pipetting with a Pasteur pipette, and add 2 or 3 drops of this suspension to the middle of the warmed agar plate. Spread the bacteria on the surface of the agar with a bacteriological loop.
3. Inoculate the specimen on the center of the agar plate as described below.
   - A. For CSF samples, centrifuge the CSF at 250 × *g* for 10 min. With a sterile serologic pipette, carefully transfer all but 0.5 ml of the supernatant to a sterile tube and store at 4°C (for possible future use). Mix the sediment in the rest of the fluid, and, with a Pasteur pipette, place 2 or 3 drops in the center of the nonnutrient agar plate that has been precoated with bacteria. After the fluid has been absorbed, seal the plates with a 5- to 6-in. length of 1-in.-wide Parafilm strip. Incubate the plate inverted at 37°C in room air. Using a wax pencil or laboratory marker, you may want to make a circle on the underside of the plate to indicate exactly where the specimen was inoculated onto the agar.
   - B. For tissue samples, triturate a small piece of the tissue (brain, lung, skin abscess, corneal biopsy, or similar specimens) in a small quantity (ca. 0.5 ml) of ameba saline. Process as above. Corneal smear, ear discharge material, etc., may be placed directly on the agar surface. Incubate central nervous system tissues at 37°C (room air) and tissues from other sites at 30°C.
   - C. Water samples of 10 to 100 ml may be processed to isolate these amebae. First, filter the water sample through three layers of sterile gauze or cheesecloth to remove leaves, dirt, etc. Next, either (i) filter the sample through a sterile 5.0-µm cellulose acetate membrane (47 mm in diameter), invert the membrane over a nonnutrient agar plate precoated with bacteria, seal, and incubate the plates as above; or (ii) centrifuge the water sample for 10 min at 250 × *g*. Aspirate the supernatant, suspend the sediment in about 0.5 ml of ameba saline, and deposit this suspension in the center of the nonnutrient

agar plate precoated with bacteria. Seal and incubate the plate at 37°C as before.

D. For soil samples, mix about 1 g of the soil sample with enough ameba saline (ca. 0.5 to 1 ml) to make a thick slurry. Inoculate this slurry in the center of the nonnutrient agar plate precoated with bacteria, and incubate as above.

E. For contact lens solutions, small volumes (ca. 1 to 2 ml) may be inoculated directly onto the nonnutrient agar plates precoated with bacteria. Larger volumes (2 to 50 ml) should be centrifuged as in step 3, and the sediment should be inoculated onto the center of the nonnutrient agar plate and incubated as above.

4. Using the low-power (10×) objective, examine the plates microscopically for amebae (cysts or trophozoites) every day for 10 days. Thin linear tracks (areas where amebae have ingested bacteria) might also be seen. If amebae are seen, circle that area with a wax pencil, carefully remove the Parafilm seal in a biological safety cabinet, open the lid of the petri dish, and carefully cut out the marked area from the agar with a spatula that has been heated to red hot and cooled before use to prevent contamination. Transfer the piece face down onto the surface of a fresh agar plate coated with bacteria, seal the plate with Parafilm, and incubate as before. *Naegleria* and *Acanthamoeba* spp. can be cultured by this method and, with periodic transfers, can be maintained in the laboratory indefinitely. In lieu of subcultures, the organisms can also be frozen for long-term storage. Under the microscope, the amebae resemble small uneven spots; observation for several seconds may reveal organism motility. After 4 to 5 days of incubation, the amebae begin to encyst and both trophozoites and cysts are visible. Unfortunately, *Balamuthia* spp. cannot be grown by using this system; they can be grown by using tissue culture methods on monkey kidney or lung fibroblast cell lines.

5. The enflagellation experiment is carried out as follows.

A. Examine the plates every day for signs of amebae. If present, amebae will feed on bacteria, multiply, and cover the entire surface of the plate within a few days. Once the food supply is exhausted, the amebae will differentiate into cysts.

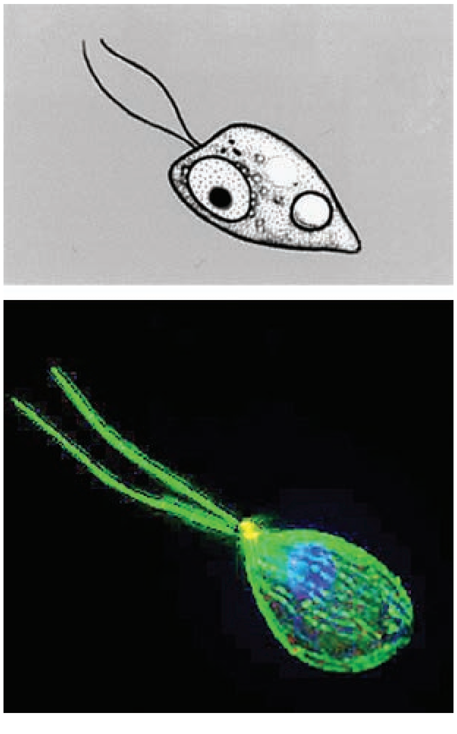
B. Use a wax pencil to mark the area containing a large number of amebic trophozoites.

C. Using a bacteriological loop, scrape the surface of the agar at the marked area and transfer several loopfuls of the scraping to a sterile tube containing about 2 ml of sterile distilled water. Alternately, flood the surface of the agar plate with about 10 ml of sterile distilled water, gently scrape the agar surface with a loop, transfer the liquid to a sterile tube, and incubate at 37°C.

D. Periodically examine the tube with an inverted microscope for the presence of flagellates.

a. *N. fowleri*, the causal agent of primary amebic meningoencephalitis, undergoes transformation to a pear-shaped flagellate, usually with two flagella but occasionally with three or four flagella; the flagellate stage is a temporary nonfeeding stage and usually reverts to the trophozoite stage (Fig. 8.2). *N. fowleri* trophozoites are typically ameba-like and move in a sinuous way. They are character-

Figure 8.2 *Naegleria fowleri* flagellate stage. (Upper) When placed in distilled water (enflagellation test), *N. fowleri*, the causal agent of primary amebic meningoencephalitis, undergoes transformation to a pear-shaped flagellate, usually with two flagella but occasionally with three or four flagella; the flagellate stage is a temporary nonfeeding stage and usually reverts to the trophozoite stage. (Lower) *Naegleria* flagellate stage (microtubules are highlighted in green, basal bodies in red, and DNA is stained blue). (Courtesy of Lillian Fritz-Laylin; from http://genome.jgi-psf.org/Naegr1/Naegr1.home.html.)
doi:10.1128/9781555819002.ch8.f2



ized by a nucleus with a centrally located, large nucleolus. The trophozoites are also characterized by the presence of a contractile vacuole that appears once every 45 to 50 s and discharges its contents. The contractile vacuole looks like a hole or a dark depression inside the trophozoite and can be easily seen when examining the plate under the 10× or 40× objective. When the food supply is exhausted, *N. fowleri* trophozoites differentiate into spherical, smooth-walled cysts.

b. In contrast, *Acanthamoeba* spp., which cause keratitis and granulomatous amebic encephalitis, do not transform into the flagellate stage. *Acanthamoeba* trophozoites are characterized by the presence of fine, thorn-like processes that are constantly extended and retracted. The trophozoites produce double-walled cysts characterized by a wrinkled outer wall (ectocyst) and a polygonal, stellate, oval, or even round inner wall (endocyst). The trophozoites are also characterized by the presence of the contractile vacuole, which disappears and reappears at regular intervals (45 to 50 s).

c. The cysts of both *Acanthamoeba* and *Naegleria* spp. are uninucleate.

d. Immunofluorescence and immunoperoxidase tests with monoclonal or polyclonal antibodies (available at the Centers for Disease Control and Prevention) are helpful in differentiating *Acanthamoeba* and *Balamuthia* spp. in fixed tissue.

Quality Control for Pathogenic Free-Living Amebae

The following control strains should be available when using these cultures for clinical specimens: ATCC 30010 (*Acanthamoeba castellanii*) and ATCC 30215 (*N. fowleri*). ATCC strains of *E. coli* or *E. aerogenes* are not necessary. Any routine clinical isolate or stock organism is acceptable.

1. Check all reagents and media (ameba saline, distilled water, and nonnutrient agar plates) each time they are used or periodically (once a week).
   A. The media and all solutions should be free of any visible signs of precipitation and bacterial and/or fungal contamination.
   B. Examine the nonnutrient agar plates under the 4× objective of an inverted or binocular microscope, and make sure that no fungal contamination has occurred.
2. Maintain stock cultures of *A. castellanii* and *N. fowleri* at 25°C.
   A. Transfer stock cultures monthly, using nonnutrient agar plates prepared with Page's ameba saline and the bacterial overlay of *E. coli*.
   B. *N. fowleri* measures 10 to 35 μm and demonstrates an eruptive locomotion by producing smooth hemispherical bulges. The cyst produces smooth (7- to 15-μm) walls. The flagellate stage does not have a cytostome.
   C. *A. castellanii* is 15 to 45 μm and produces fine, tapering, hyaline projections called acanthapodia. It has no flagellate stage but produces a double-walled cyst with a wrinkled outer wall (10 to 25 μm).
   D. Trophozoites of *Naegleria* and *Acanthamoeba* spp. are uninucleate, characterized by a large, dense central nucleolus.
   E. For staining, a slide prepared from a stock strain of amebae is run in parallel with the patient slide. Staining results are acceptable when the control amebae stain well.
   F. Plate both stock cultures onto fresh media, and incubate at 37°C parallel with the patient culture. Culture results are acceptable when growth appears by day 7.
   G. Run *N. fowleri* in parallel with the patient culture being observed for enflagellation. The test results are acceptable when free-swimming, pear-shaped flagellates with two flagella are observed in 2 to 24 h on the control slide.
3. Depending on use, the microscope(s) may have to be calibrated (within the last 12 months), and the original optics used for the calibration should be in place on the microscope(s). The calibration factors for all objectives should be posted on the microscope for easy access.

Note For patient specimens, if a plate is positive for amebae and the amebae transform into flagellates, the specimen should be reported as positive for *N. fowleri*. If the amebae do not transform into flagellates even after overnight incubation, if the trophozoites possess the characteristic acanthapodia, if they show a large centrally placed nucleolus in the nucleus on trichrome stain, and if the trophozoites differentiate into the characteristic double-walled cysts, the specimen should be reported as positive for *Acanthamoeba* sp. For contact lens solution, if the plates are positive for amebae and the amebae do not transform into flagellates but differentiate into cysts with a wrinkled outer ectocyst and an inner stellate, polygonal, oval, or round endocyst, the specimen should be reported as positive for *Acanthamoeba* sp. *Naegleria* spp. have not been isolated from contact lens solutions; however, small amebae (e.g., hartmannellid or vahlkampfiid amebae, which produce smooth-walled cysts), probably contaminants, have occasionally been isolated from these solutions. Plates inoculated with water samples are usually positive for many genera and species of small free-living amebae (freshwater is their normal habitat). Therefore, the sample should be reported as positive for small free-living amebae. The physician should be notified immediately if patient specimens are positive for *Acanthamoeba* or *Naegleria* spp.

Most patient specimens, especially CSF, should be examined microscopically as soon as they arrive in the laboratory. However, if the specimen is very clear with no visible sediment, it should be cultured without being examined microscopically.

1. After centrifugation, remove a small drop of the CSF sediment, place it on a microscope slide, cover it with a no. 1 coverslip, seal the edges of the coverslip with Vaspar (optional), and examine it immediately with the 10× or 40× objective (phase-contrast/differential interference contrast optics are preferred). If bright-field microscopy is used, reduce the illumination by adjusting the iris diaphragm.
   A. The *N. fowleri* trophozoite is highly motile and can be identified by its sinuous movement. A warmed penny may be applied to the bottom surface of the slide to activate the movement of the trophozoite. Occasionally, a flagellate is seen traversing the field.
   B. *Acanthamoeba* trophozoites are rarely seen in the CSF. If present, they may be recognized by their characteristic acanthapodia, which are constantly extended and retracted. Both amebae, especially *Acanthamoeba* spp., may be recognized by the contractile vacuole.
2. Contact lens care solutions (opened) can be processed and examined like CSF. Testing of unopened commercial lens care solutions should be handled by the FDA.
3. If very small amounts of tissue are received, they should be reserved for culture. If the specimen received is visibly contaminated with bacteria and/or fungi, the tube containing the specimen should be placed in an ice-water bath for 3 min prior to centrifugation. This causes any trophozoites attached to the walls of the tube to come off and drop into the fluid prior to centrifugation.
4. Material from the surface of a positive agar plate can be removed, fixed, and stained with trichrome for microscopic examination at a higher magnification (×1,000). Results obtained with wet mounts should always be confirmed by performing permanent stained smears (trichrome, iron hematoxylin) for nuclear characteristics to differentiate the amebae from host cells. Organisms may not be recovered if appropriate centrifugation speeds and times are not used.

Liquid Culture Media for Pathogenic Free-Living Amebae

Although *Acanthamoeba* spp. can be grown in serum-free liquid medium, *N. fowleri* requires the addition of fetal calf serum or brain extract to grow in a liquid medium. Peptone-yeast extract-glucose medium for *Acanthamoeba* spp. and Nelson's medium for *N. fowleri* are described below.

Peptone-Yeast Extract-Glucose (PYG) Medium for *Acanthamoeba* spp.

Proteose peptone (Difco)	20.0 g
Yeast extract (Difco)	2.0 g
Magnesium sulfate ($MgSO_4$)	0.980 g
Calcium chloride ($CaCl_2$)	0.059 g
Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	1.0 g
Ferric ammonium sulfate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$)]	0.02 g
Potassium phosphate, monobasic (KH_2PO_4)	0.34 g
Sodium phosphate, dibasic ($Na_2HPO_4 \cdot 7H_2O$)	0.355 g
Glucose	18.0 g
Distilled water to	1,000.0 ml

Final pH is 6.5 ± 0.2.

1. Dissolve all ingredients except $CaCl_2$ in about 900 ml of distilled water in a bottle or flask with a magnetic stirrer.
2. Add $CaCl_2$ while stirring.
3. Bring the volume to 1,000 ml with distilled water.
4. Dispense into 16- by 125-mm screw-cap tubes, 5 ml per tube.
5. Autoclave at 121°C for 15 min.
6. When tubes have cooled, label the tubes as *Acanthamoeba* medium with the preparation date and an expiration date of 3 months.
7. Store at 4°C.

Nelson's Medium for *N. fowleri*

Panmede (ox liver digest) (Difco)	1.0 g
Glucose	1.0 g
10× Page's ameba saline (p. 163)	100.0 ml
Double-distilled water to	900.0 ml

1. Add ameba saline to distilled water to make 1× ameba saline.
2. Dissolve the ingredients in ameba saline with a magnetic stirrer.

3. Dispense into 16- by 125-mm screw-cap tubes, 10 ml per tube.
4. Autoclave for 15 min at 15 lb/in.^{2}.
5. Cool, and label as Nelson's medium with the preparation date and an expiration date of 3 months.
6. Store at 4°C.
7. Add 0.2 ml of heat-inactivated fetal calf serum to each tube before inoculating with the amebae.

Cell Culture

Acanthamoeba, *Balamuthia*, *Naegleria* spp., and *Sappinia* spp. can be inoculated onto a number of different mammalian cell lines. The organisms grow very well and demonstrate cytopathic effects, similar to those seen in routine viral cultures. However, most laboratories do not offer these methods on a routine basis.

Standard Commercial Media for Growth and Isolation of *Acanthamoeba* spp.

Several studies have reported on the use of standard commercial media used for growth and isolation of *Acanthamoeba* spp. Buffered charcoal-yeast extract agar (BCYE) is an excellent commercially available culture medium for the recovery of *Acanthamoeba* spp. Good trophozoite recovery was obtained using BBL Trypticase soy agar (TSA) with 5% rabbit blood, TSA with 5% horse blood, and Remel TSA with 5% sheep blood. BBL TSA with 5% horse blood or 5% rabbit blood yielded good recovery of cysts. Nonnutrient agar with either live or dead bacteria yielded good recovery of trophozoites; however, live bacteria may be required for good cyst recovery.

Blastocystis spp. (*Blastocystis hominis*)

Blastocystis is a single-celled enteric protozoan that has a worldwide distribution. *Blastocystis* belongs to the phylum Stramenopila, is found in humans and animals, and is the most common parasite isolated from human stool samples throughout the world. Rates of infection vary from <5% in developed countries to >50% in developing countries. Specimens include stool material, mucus, or a combination of the two. The clinical specimen(s) should be no more than 24 h old; a maximum of 2 to 3 h is recommended. Xenic or monoxenic laboratory cultures of *Blastocystis* isolates, which are cultures of *Blastocystis* cells grown in association with nonstandardized or single known species of microorganisms, respectively, can be maintained in TYGM-9 medium or Boeck and Drbohlav's inspissated egg medium (see below) (12).

Tryptone/Yeast Extract/Glucose/Methionine) TYGM-9 Xenic Medium

See TYSGM-9 Medium for *Entamoeba histolytica* earlier in this chapter.

Diphasic LE Medium (*Blastocystis* spp.)

LE medium is the NIH modification of Boeck and Drbohlav's medium.

Locke's Solution

NaCl	8.0 g
$CaCl_2$	0.2 g
KCl	0.2 g
$MgCl_2$	0.01 g
Na_2HPO_4	2.0 g
$NaHCO_3$	0.4 g
KH_2PO_4	0.3 g
Distilled water	1,000 ml

Autoclave for 15 min at 121°C under a pressure of 15 lb/in.^{2}. Cool to room temperature, and remove any precipitate by filtration (Whatman no. 1 paper). Reautoclave to sterilize.

Note The overlay can also be prepared using 90% PBS, 9% sterile horse serum, 1% or 20% [wt/vol] bacteriologic peptone, 1 mg of rice starch, and 500 µl of penicillin-streptomycin solution (12).

Preparation of Complete Medium

1. To prepare the egg slant, surface sterilize fresh hen eggs by flaming in 70% ethanol and break into a graduated cylinder.
2. Add 12.5 ml of Locke's solution per 45 ml of egg.
3. Emulsify in a Waring-type blender, and filter through gauze into a flask.
4. Place under vacuum to draw out all air bubbles.
5. Add 5-ml amounts of the emulsified egg to standard culture tubes (16 by 125 mm), and autoclave at 100°C for 10 min with the tubes at an angle that produces a 12- to 15-mm (ca. 0.5-in.) butt. The resulting egg slants should be free of bubbles.
6. Cool to room temperature, overlay slants with 6 ml of Locke's solution, and autoclave for 15 min at 121°C under a pressure of 15 lb/in.^{2}.
7. Let the slants cool to room temperature, tighten the caps, and refrigerate the slants for up to 6 months.

The culture should be examined every 2 days for 1 week (phase-contrast microscopy is recommended) (12). Another option is to use reduced light with the condenser lowered to provide more contrast. If parasites are seen in the sediment, the material can be placed in a fecal preservative for staining and examination using the oil immersion objective (100× oil immersion objective).

Pathogenic Flagellates

Trichomonas vaginalis

Specimens from women may consist of vaginal exudate collected from the posterior fornix on cotton-tipped applicator sticks or genital secretions collected on polyester sponges. Specimens from men can include semen, urethral samples collected with polyester sponges, or urine. Urine samples collected from the patient should be the first voided specimen in the morning. It is critical that clinical specimens be inoculated into culture medium as soon as possible after collection (23–26). Although collection swabs can be used, there are often problems with specimens drying prior to culture; immediate processing is mandatory for maximum organism recovery. The culture method is considered to be the most sensitive for the diagnosis of trichomoniasis; however, because of the time and effort involved, many laboratories have changed to the monoclonal antibody detection kits or more automated molecular methods. Another approach would be to use the plastic envelope method for *Trichomonas vaginalis* (InPouch TV [BIOMED Diagnostics, San Jose, CA]), a simplified technique for the transport and culture that is illustrated in Fig. 8.3 and 8.4. Because of its long shelf life, relatively low expense, and high sensitivity, studies confirm that the pouch system provides a good diagnostic culture method for *T. vaginalis* (27, 28). It has been found to be more sensitive than either Diamond's or Trichosel medium (27).

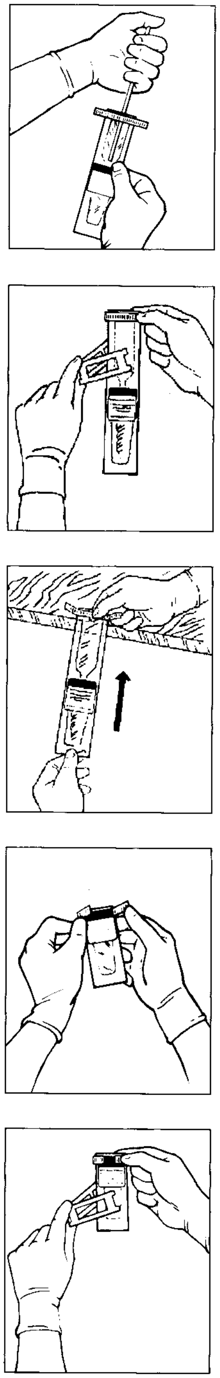


Figure 8.3 Illustration of the InPouch TV culture system for *Trichomonas vaginalis*. From top to bottom: (1) introduction of the specimen into the upper chamber containing a small amount of medium; (2) application of a plastic holder for microscope viewing prior to expressing medium into the lower chamber (optional); (3) transfer of a small amount of medium in the upper chamber to the lower chamber; (4) rolling down the upper chamber and sealing it with the tape; (5) plastic viewing frame used to immobilize the medium in the pouch for examination under the microscope. doi:10.1128/9781555819002.ch8.f3

Lash's Casein Hydrolysate-Serum Medium

Lash's casein hydrolysate-serum medium is a culture medium used to diagnose *T. vaginalis*.

Lash's Casein Hydrolysate-Serum Medium

Casamino Acids (Difco)	14.0 g
Maltose	1.5 g
Dextrose	2.0 g
Sodium lactate (60% solution)	0.5 g

Dissolve in 500 ml of distilled water. To this solution, add:

NaCl	6.0 g
KCl	0.1 g
$CaCl_2$	0.1 g

1. Adjust the pH to 6.0 with concentrated phosphoric acid. Dispense in 5-ml aliquots, plug the tubes, and autoclave at 121°C for 15 min.
2. Prepare serum solution:

Whole beef blood serum	200 ml
Distilled water	300 ml
$NaHCO_3$	0.1 g

3. Sterilize the serum solution by filtration.
4. The complete medium contains 5 ml of serum solution and 5 ml of basic solution for each tube.

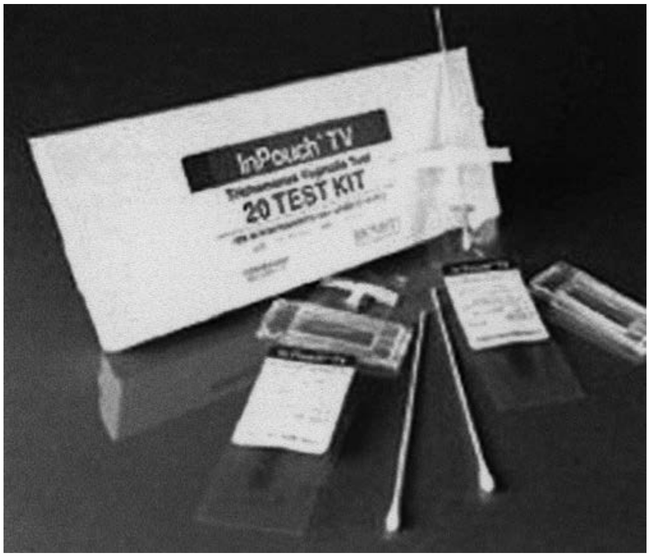


Figure 8.4 InPouch TV culture system for *Trichomonas vaginalis*. Note the pouch, swabs, and plastic pouch holder for microscopic examination of the pouch contents.
doi:10.1128/9781555819002.ch8.f4

5. Incubate the cultures at 37.5°C, and examine 24 and 48 h after inoculation.

T. vaginalis has been studied for a number of years with agar plate cultures for both cloning and diagnosis (2–4). Although broth and agar culture for *T. vaginalis* are successful, many laboratories have transitioned to molecular based methods. Many media are available for the isolation of *T. vaginalis*; some of these can be purchased commercially and have a relatively long shelf life.

CPLM (Cysteine-Peptone-Liver-Maltose) Medium

Ringer's solution

Sodium chloride (NaCl)	0.6 g
Sodium bicarbonate ($NaHCO_3$)	0.01 g
Potassium chloride (KCl)	0.01 g
Calcium chloride ($CaCl_2$)	0.01 g
Double-distilled water to	100.0 ml

Dissolve the ingredients in the order listed, and bring the volume up to 100.0 ml with distilled water.

Liver infusion

Double-distilled water	330.0 ml
Bacto Liver Infusion powder (Difco)	20.0 g

1. Place the distilled water in a large beaker.
2. Add the liver infusion powder.
3. Infuse for 1 h at 50°C.
4. Raise the temperature to 80°C for 5 min to coagulate the protein.
5. Filter through a Whatman no. 1 paper with a Büchner funnel.

Methylene blue solution

Methylene blue	0.5 g
Glass-distilled water	100.0 ml

Mix well until dissolved.

CPLM (Cysteine-Peptone-Liver-Maltose) Complete Medium

Bacto Peptone (Difco)	32.0 g
Bacto Agar (Difco)	1.6 g
Cysteine HCl	2.4 g
Maltose	1.6 g
Bacto Liver Infusion (Difco)	320.0 ml
Ringer's solution	960.0 ml

1. With a magnetic stirrer, mix Ringer's solution and the liver infusion in a large beaker.
2. Add peptone, maltose, cysteine HCl, and agar in that order, and heat the mixture until dissolved.
3. Add 0.7 ml of aqueous methylene blue.
4. Adjust the pH to 5.8 to 6.0 with 1 N NaOH or 1 N HCl.
5. Dispense 8-ml volumes into culture tubes.
6. Autoclave at 121°C for 15 min.
7. Aseptically add 2 ml of human serum, heat inactivated at 56°C for 30 min and cooled, per tube. Horse serum is recommended as a replacement for human serum, particularly when considering safety issues such as handling human blood and blood products.
8. Label as CPLM medium with the preparation date.
9. Store at room temperature. Use as long as the amber zone indicating an anaerobic condition persists.

Diamond's TYM (Trypticase-Yeast Extract-Maltose) Complete Medium

Trypticase (BBL)	20.0 g
Yeast extract (BBL)	10.0 g
Maltose	5.0 g
L-Cysteine HCl	1.0 g
L-Ascorbic acid	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	0.8 g
Potassium phosphate, monobasic (KH_2PO_4)	0.8 g
Bacto Agar (Difco)	0.5 g
Double-distilled water	900.0 ml

1. Dissolve the buffer salts in the distilled water with a magnetic stirrer.
2. Add the remaining ingredients except the agar, in the order given, one at a time until dissolved.
3. Adjust the pH to 6.0 with 1 N HCl.
4. Add agar, and heat to dissolve.
5. Autoclave at 121°C for 15 min.

6. Cool to 45°C, and add 100 ml of bovine, sheep, or horse serum that has been inactivated for 30 min at 56°C.
7. Aseptically dispense 10-ml volumes to 16- by 125-mm screw-cap tubes.
8. Label as TYM medium with the preparation date and an expiration date of 10 days.
9. Store at 4°C.

Hollander's Modification of TYM Complete Medium

Hollander's modification of TYM complete medium differs from TYM medium by the replacement of cysteine with additional ascorbic acid and the addition of potassium chloride, potassium carbonate, and ferrous sulfate (2).

Casein digest peptone (Trypticase, BBC)	20.0 g
Yeast extract	10.0 g
Maltose	5.0 g
Ascorbic acid	1.0 g
Potassium chloride (KCl)	1.0 g
Potassium bicarbonate	1.0 g
Potassium phosphate, monobasic (KH_2PO_4)	1.0 g
Potassium phosphate, dibasic (K_2HPO_4)	0.5 g
Ferrous sulfate	0.1 g
Distilled water	600 ml

Proceed as described above for Diamond's TYM complete medium.

Note Hollander also includes agar to 0.05%.

Diamond's Complete Medium (Modified by Klass)

Trypticase (BBL)	24.0 g
Yeast extract (BBL)	12.0 g
Maltose	6.0 g
Cysteine HCl	1.2 g
Ascorbic acid	0.24 g
Double-distilled water	900.0 ml

1. Dissolve the ingredients one at a time in the order given.
2. Adjust the pH to 6.0 with 1 N HCl or 1 N NaOH.
3. Dispense in 12.5-ml aliquots to 16- by 125-mm screw-cap tubes.
4. Autoclave at 121°C for 15 min.
5. When cool (50°C), add 1 ml of sterile inactivated horse serum and 0.5 ml of antibiotic mixture to each tube.
6. Label as modified TYM medium with the date of preparation and an expiration date of 3 weeks.

Antibiotic Mixture

Sodium penicillin G	1,000,000 U
Streptomycin sulfate	1,000,000 µg
Fungizone (amphotericin B)	2,000 µg
Sterile double-distilled water	50.0 ml

1. Mix thoroughly.
   Concentration of stock solution is:

Penicillin	20,000 U/ml
Streptomycin	20,000 µg/ml
Fungizone	40 µg/ml

2. Dispense 1 ml of the antibiotic mixture into sterile screw-cap vials or sterile cryovials.
3. Label as antibiotic solution with the date of preparation and an expiration date of 1 year.
4. Store at 220°C in cryoboxes.

Serum Substitutions

Bovine, sheep, or horse serum can be substituted in the step involving CPLM complete medium.

Axenic Culture

1. Inoculation of culture medium.
   A. Remove tubes containing culture medium from 4°C, and incubate at 37°C for 1 to 2 h.
   B. Vigorously shake the cotton-tipped portion of the applicator stick containing the patient specimen in the medium, and then break off the tip with sterile forceps and drop it into the medium.
   C. If the material is collected on polyester sponges, drop the sponges into the medium and shake the tube.
   D. Centrifuge urine samples for 10 min at 250 × *g*, aspirate the supernatant, and inoculate the sediment into the medium.
   E. Examine the tubes daily for several days, and subculture if necessary. To subculture, first shake the tube to disperse the organisms uniformly, remove about 1 to 2 ml, and inoculate into a warmed, fresh tube.
   F. Incubate the tubes in a slanted position (45° angle).
   G. Incubate the control tubes and those containing patient material for at least 72 to 96 h.
   H. Examine the entire length of the tube. If the specimen is positive, *T. vaginalis* will be found freely swimming or attached to the tube walls.
   I. Do not report negative results until 96 h.
2. To maintain stock cultures:
   A. With an inverted microscope, examine stock culture tubes of *T. vaginalis* for any signs of bacterial contamination. Select one or more tubes showing good growth. Since the tubes are incubated in a slanted position, usually at an angle of 45°, a thick button of organisms is seen at the bottom of the tube. Gently invert the tube once or twice to disperse the tricho-

monads uniformly, and examine the tubes again. A large number of the organisms should be freely swimming, and a few will be attached to the tube walls.

B. Immerse the tubes in a bucket of ice-cold water for about 5 to 10 min to dislodge the trichomonads from the tube walls. Invert the tubes several times to distribute the organisms.

C. With a sterile Pasteur pipette, remove about 1.0 to 1.5 ml; inoculate 0.5 to 1.0 ml into a fresh tube. Inoculate the rest of the fluid into nutrient agar, brain heart infusion, and thioglycolate broth for routine monitoring of bacterial contamination. Inoculate several additional tubes this way, and incubate the cultures slanted at a 45° angle at 37°C as before.

D. If growth is poor and only few organisms are present along with a lot of debris, centrifuge the tube at 250 × *g* for 10 min, aspirate the supernatant, transfer the sediment to a fresh tube, and incubate as before.

Quality Control for Pathogenic Flagellates (*T. vaginalis*)

ATCC 30001 (*T. vaginalis*) should be available as a control strain when these cultures are used for clinical specimens.

1. Check all reagents and media (at least once a week). All media, including Ringer's solution, should be free of any signs of precipitation and bacterial and/or fungal contamination.
2. Depending on use, the microscope(s) may have had to be calibrated within the last 12 months, and the original optics used for the calibration should be in place on the microscope(s). The calibration factors for all objectives should be posted on the microscope for easy access.
3. Maintain stock cultures of ATCC 30001 (*T. vaginalis*).
   A. Transfer stock cultures weekly.
      a. Stock organisms should always be cultured at the same time a patient specimen is inoculated into culture medium.
      b. If the stock organisms multiply and remain viable during the 96 h, patient results can be reported.
   B. Stain
      a. A slide prepared from a stock strain of *T. vaginalis* is run in parallel with the patient slide.
      b. Staining results are acceptable when the control organisms stain well.
4. Control organisms must be cultured each time - a patient specimen is inoculated into the culture medium.

Note If no trophozoites are seen after 4 days of incubation, discard the tubes and report the culture as negative. Results of patient specimens should not be reported as positive unless control cultures are positive. Cultivation is the most sensitive method for the diagnosis of trichomoniasis. Every effort, therefore, must be made to inoculate patient materials into culture medium. However, since this method may take as long as 3 to 4 days and the patient materials occasionally contain nonviable organisms, it is imperative that microscopic examination of wet smears and/or stained smears (Giemsa) also be performed.

Axenic Cultivation of *T. vaginalis* in a Serum-Free Medium

Mammalian serum or bovine serum albumin is required for *T. vaginalis* grown under axenic conditions. However, these components inhibit several biological properties of these parasites. PACSR is a serum replacement, free of bovine serum albumin, which is used for axenic cultivation of *E. histolytica*. Studies indicate that PACSR added to several of the standard media used for the cultivation of *T. vaginalis* can support growth in the absence of serum (29).

Dientamoeba fragilis

Dientamoeba fragilis is a protozoan pathogen of the human gastrointestinal tract that was originally classified as an amoeba. Later studies confirmed its relationship with trichomonads. The approach to *in vitro* culture is not new; however, some excellent improvements have recently been developed (30, 31). Currently, no axenic cultures of *D. fragilis* exist. *D. fragilis* will grow happily with a support flora consisting mostly of *E. coli*. Slight variations in the species of prokaryotic support flora present within *D. fragilis* cultures are unlikely to exhibit any significant effect on growth. A modified Earle's Balanced Salt Solution (EBSS) containing cholesterol, ferric ammonium citrate, and rice starch can be regarded as a superior liquid overlay that can be used along with the Loeffler's serum slope for culture of *D. fragilis* under anaerobic conditions.

Loeffler's Serum Slants (30)

Heat-inactivated horse serum	700 ml/liter
Glucose	2.5 g/liter
Nutrient broth No.2	6.25 g/liter in distilled water

1. Aliquot 5 ml into tubes, slant, and inspissate in 80°C drying oven.
   Note If the slants are left too long at 80°C, *Dientamoeba* will not grow. Slants should be allowed to cool IMMEDIATELY after they solidify.
2. Add approximately 2 mg of rice starch into the bottom of each slant.

3. Overlay each slant with 5 ml of PBS.
4. After inoculation with fresh stool (pea size), incubate at 42°C under microaerophilic conditions (6% O_2, 7.2% CO_2, 3.6% H_2, 83.3% N_2).

Earle's Balanced Salt Solution Overlay

Another overlay that has proven to be very effective is EBSS, pH 7.8. EBSS provides the highest trophozoite numbers and contains a variety of inorganic salts, as well as sodium hydrogen carbonate ($NaHCO_3$) and glucose. The inorganic salts in the medium help to maintain the osmolarity as well as providing a range of divalent cations needed for cell metabolism. $NaHCO_3$ maintains the buffering capacity in the anaerobic *in vitro* culture and glucose provides an additional energy source in addition to the rice starch. This saline solution has also been used with good results when supplemented with 40 mg/liter ferric ammonium citrate and 50 mg/liter cholesterol (31).

NaCl	6.8 g
KCl	0.4 g
$CaCl_2$	0.2 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$NaH_2PO_4 \cdot 7H_2O$	0.14 g
$NaHCO_3$	2.2 g
Glucose	1.0 g
Distilled water	1,000 ml

Autoclave and store at room temperature.

Cryopreservation (30)

Dimethyl sulfoxide	1.375% (vol/vol) in
PBS	single strength

1. Pool four culture tubes of Loeffler's medium containing viable organisms.
2. Centrifuge at 500 × *g* for 5 min; discard supernatant fluid.
3. Add 1 ml of PBS to the pellet and invert tube to obtain even cell suspension.
4. Count the cells present.
5. Dilute quantified cell suspensions in PBS to a final concentration of 10^6 trophozoites/μl.
6. Plate tubes overnight at –80°C freezer.
7. Remove tubes from the –80°C freezer the following morning and place in a liquid nitrogen freezer.

Flagellates of Blood and Tissue

The first types of media used to culture the blood and tissue flagellates, which are still useful for establishment of cultures, were undefined and contained a complex mixture of ingredients. Improvements led to semidefined formulations that included tissue culture media as a base and, as a next step, addition of tissue culture cells as a feeder layer to promote parasite growth. Newer developed media are completely defined, having replaced the feeder cells with various supplements. Serum, a variable component of the media, can now be replaced by various serum substitutes. Fully defined formulations are available for the cultivation of many of these organisms (8).

For the hemoflagellates found in the bloodstream, probably the most direct means of diagnosis is by examination of a stained blood smear under the microscope (any blood stain is acceptable). However, if isolation of the parasite is required for confirmation, culture can be used. The easiest approach involves the use of Novy-MacNeal-Nicolle (NNN) medium. Because of its relatively short shelf life (2 to 4 weeks with refrigeration) and the infrequent need for culture, most clinical laboratories would not routinely stock NNN medium. However, the Centers for Disease Control and Prevention (Division of Parasitic Diseases, Atlanta, GA) can provide culture services, and in other parts of the world, major reference laboratories may provide a similar service. The procedure for isolating *Leishmania* spp. from patients with suspected cases of cutaneous, mucocutaneous, and visceral leishmaniases is basically similar to that for isolating *Trypanosome* spp. The amastigote found within host cells, or the promastigote that develops from the amastigote in culture, would be confirmatory. Punch biopsy specimens or needle aspirates serve as the inocula for NNN medium or Schneider's *Drosophila* medium with 30% fetal bovine serum. Other procedures such as splenic puncture and liver biopsy can also yield material but are invasive and less likely to be performed (8).

Specimens for culturing *Leishmania* spp. may consist of aspirates, scrapings, or biopsy material from skin lesions of patients with cutaneous leishmaniasis; bone marrow aspirates or, more rarely, splenic aspirates from visceral leishmaniasis patients; or normal skin biopsy specimens, lymph node aspirates, or pieces of liver and spleen from suspected or potential wild- or domestic-animal reservoirs. Ensure that the skin surrounding the ulcer is thoroughly cleaned and swabbed with 70% alcohol (sterile saline is not acceptable as a cleansing agent) and allowed to dry before the sample is removed. Also ensure that alcohol does not get into the ulcerated area or broken skin. A punch biopsy specimen taken from the advancing margin of the lesion is often recommended. If skin biopsy fragments are placed in transport medium (RPMI medium supplemented with 10% fetal calf serum) maintained at ambient temperature, delays in transit through the mail will still not prevent recovery of the organisms. In one study, after being received in the laboratory (transit time as long as 3 to 17 days), the biopsy specimens were ground in sterile saline and inoculated into NNN culture tubes. The tubes were incubated at 25°C and subcultured every week until the fifth week. Cultures were positive in 9 of 16 cases in all seasons and for three different *Leishmania* species. Thus, delayed culture can still yield valuable results from biopsy specimens obtained under field conditions (32).

It is imperative that only a few drops of bone marrow juice or spleen aspirate be inoculated into tubes. Inoculate several tubes with a few drops each rather than inoculating a single tube with a large volume (1 to 2 ml), since the serum in the specimen may contain leishmanicidal or inhibitory factors that prevent the growth of organisms. Alternatively, bone marrow juice may be centrifuged for 10 min at 250 × *g* and the sediment may be washed in 0.85% saline by centrifugation and then inoculated into culture tubes. Buffy coat from the blood sample rather than whole blood should be inoculated. Because the leishmanias are fastidious organisms and not all isolates may grow in any one medium, it is imperative that at least two media be used; for example, use NNN or modified Tobie's medium and Schneider's *Drosophila* medium.

Specimens for culturing *Trypanosoma cruzi* may consist of blood or the gut contents of the triatomid bug. It is advisable to use two different media such as liver infusion-tryptose (LIT) and NNN for the initial isolation of *T. cruzi*. Once growth is established, use the medium in which best growth is obtained for subculture. According to James Sullivan, LIT medium, when used as an overlay on Tobie's slants, is excellent for isolation and diagnosis (8). The major culture form is the epimastigote; occasionally, however, trypomastigotes and amastigotes are also seen.

NNN Medium (Leishmaniasis or Chagas' Disease) (33)

Bacto Agar (Difco)	1.4 g
Sodium chloride (NaCl)	0.6 g
Double-distilled water	90.0 ml

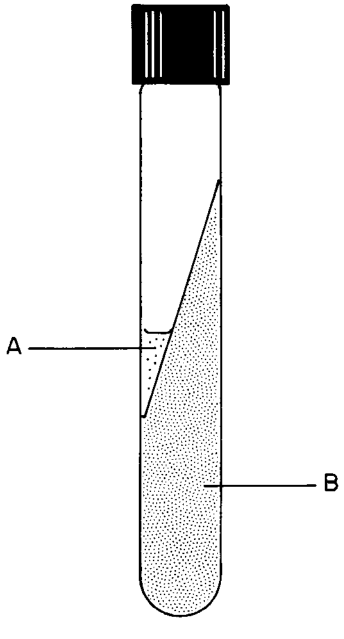
1. Mix the NaCl and agar in the distilled water in a 500-ml flask.
2. Heat the mixture until the agar melts.
3. Autoclave at 121°C for 15 min.
4. Cool to about 50°C.
5. Add 10 ml of aseptically collected, defibrinated rabbit blood.
   Note Although blood collected with EDTA as the anticoagulant can be used for routine stock culture subcultures, it may not be quite as effective as defibrinated blood in isolating organisms from patient specimens. Certainly, if defibrinated blood is not available, blood collected with EDTA as the anticoagulant can be used. Make sure that the rabbit blood is fresh (no older than 10 days). It should be aseptically collected and stored at 4°C until used.
6. Dispense 4 ml into 16- by 125-mm sterile screw-cap culture tubes.
7. Place the tubes at a 10° angle (shallow slant position) until the agar sets.
8. Immediately transfer the tubes into test tube stands, and let stand upright at 4°C so that the bottom portion of the slants is covered with the water of condensation. Rapid cooling increases the water of condensation (Fig. 8.5).
9. Label as NNN medium with the preparation date and an expiration date 3 weeks from the date of preparation.
10. Store at 4°C.

NNN Medium, Offutt's Modification (Leishmaniasis)

Blood Agar Base (Difco)	8.0 g
Double-distilled water	200.0 ml

1. Heat until the agar is dissolved in the distilled water in a 500-ml flask.
2. Autoclave at 121°C for 15 min.
3. Cool to about 50°C.
4. Add 15 ml of aseptically collected, defibrinated rabbit blood.
5. Dispense 4 ml into 16- by 125-mm sterile screw-cap culture tubes.
6. Place the tubes at a 10° angle (shallow slant position) until the agar sets.

Figure 8.5 Illustration of a tube of NNN medium, used for the culture and recovery of *Leishmania* spp. (A) Fluid condensate and tissue culture medium overlay containing the developing organisms. (B) Blood agar medium. (Illustration by Sharon Belkin.)
doi:10.1128/9781555819002.ch8.f5



7. Immediately transfer the tubes to test tube stands, and let stand upright at 4°C so that the bottom portion of the slants is covered with the water of condensation. Rapid cooling increases the water of condensation.
8. Label as Offutt's medium with the preparation date and an expiration date 3 weeks from the date of preparation.
9. Store at 4°C.

Overlay Solution (To Be Used with NNN or NNN Modified)

Sodium chloride (NaCl)	4.5 g
Double-distilled water	500.0 ml

1. Autoclave at 121°C.
2. Dispense 4 ml aseptically into 16- by 125-mm sterile culture tubes.
3. Label as 0.9% saline with the preparation date and an expiration date 3 weeks from the date of preparation.
4. Store at 4°C.

Evan's Modified Tobie's Medium (Leishmaniasis or Chagas' Disease) (33)

Beef extract (Oxoid Lab-Lemco L29)	0.3 g
Bacteriological peptone (Oxoid L37)	0.5 g
Sodium chloride (NaCl)	0.8 g
Agar (Oxoid purified)	2.0 g
Double-distilled water	100.0 ml

1. Mix all the ingredients in the distilled water in a large beaker with a magnetic stirrer.
2. Heat the mixture until the agar melts.
3. Dispense 5 ml into 16- by 125 mm screw-cap culture tubes.
4. Autoclave at 121°C for 15 min.
5. Cool to about 50°C.
6. Add 1.2 ml of aseptically collected, defibrinated horse blood.
7. Hold the tubes upright in the palm of your hand, and roll the tubes gently to mix the blood and the agar well.
8. Place the tubes at a 10° angle (shallow slant position) until the agar sets.
9. Immediately transfer the tubes into test tube stands, and let stand upright at 4°C so that the bottom portion of the slants will be covered with the water of condensation. Rapid cooling increases the water of condensation.
10. Label as Evan's modified Tobie's medium with the preparation date and an expiration date 3 weeks from the date of preparation.
11. Store at 4°C.

Overlay Solution (To Be Used with Tobie's Medium)

Potassium chloride (KCl)	0.4 g
Sodium phosphate, dibasic ($Na_2HPO4 \cdot 12H_2O$)	0.06 g
Potassium phosphate, monobasic (KH_2PO4)	0.06 g
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.185 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.1 g
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.1 g
NaCl	8.0 g
L-Proline	1.0 g
Phenol red	0.5 ml
Double-distilled water	1,000.0 ml

1. Place 750 ml of double-distilled water in a 1-liter beaker, and add the above ingredients one at a time in the order listed until dissolved. Use a magnetic stirrer.
2. Adjust the pH to 7.2 by adding slowly, while stirring, solid Tris (white, crystalline powder, $C_4H_{11}NO_3$ used for buffering capacity).
3. Bring up the volume to 1,000 ml with distilled water.
4. Dispense 100 ml into each of several screw-cap flasks or bottles.
5. Autoclave at 121°C for 15 min.
6. Label as overlay solution with the preparation date and an expiration date of 1 month.
7. Store at 4°C.

NIH Method for Trypanosomes and Leishmaniae

Solution 1

Lean beef, desiccated	25.0 g
Neopeptone	10.0 g
Agar	10.0 g
NaCl	2.5 g
Distilled water	500 ml

Solution 2 (Locke's Solution)

NaCl	8.0 g
KCl	0.2 g
$CaCl_2$	0.2 g
KH_2PO_4	0.3 g
Dextrose	2.5 g
Distilled water	1,000 ml

1. Infuse the beef and distilled water in a water bath at 37°C for 1 h. Heat for 5 min at 80°C.
2. Filter through filter paper (Whatman no. 2V).
3. Add the rest of the above ingredients (solution 1), and adjust the pH to 7.0 to 7.4 with NaOH.
4. Autoclave at 121°C for 20 min.

5. Cool to 45°C, and aseptically add 10% defibrinated rabbit blood.
6. Dispense 5-ml quantities in sterile tubes. Slant and cool.
7. Just before inoculation, overlay with 2 ml of sterile Locke's solution.

4 N Medium for Trypanosomes and Leishmaniae

The 4 N medium was adapted from the original formula described by Novy and McNeal (9, 34, 35).

4 N Medium for Trypanosomes and Leishmaniae

Agar base

Oxoid blood sugar base no. 2	40 g
Distilled water	1,000 ml

1. Mix the agar and water, and dissolve by autoclaving or steaming.
2. Dispense liquid agar in 5-ml aliquots into 30-ml screw-cap glass bottles.
3. Autoclave again if necessary for sterility.
4. When the medium has cooled to 45°C, add aseptically to each bottle approximately 1 ml of fresh rabbit blood and allow the agar to solidify in a slant.

Overlay for 4 N medium

1. Add 1 ml of Locke's (see NIH method for trypanosomes and leishmaniae) solution to each bottle containing 5 ml of agar.
2. Incubate the bottles at 37°C for 12 to 24 h to check for sterility.
3. Store the bottles at 4°C for 24 h or more before use.

Yaeger's LIT Medium (for Chagas' Disease) (33)

Liver infusion (Difco)	35.0 g
Tryptose (Difco)	5.0 g
NaCl	4.0 g
KCl	0.4 g
Sodium phosphate, dibasic ($Na_2HPO_4 \cdot 12H_2O$)	8.0 g
Glucose	2.0 g
Hemin (stock solution)	4.0 ml
Double-distilled water	1,000.0 ml

1. Add all the ingredients to the distilled water, and mix well with a magnetic stirrer until dissolved. Heat, if necessary, to dissolve all the ingredients.
2. Using a Whatman no. 42 filter paper in a Büchner funnel, filter with suction. Repeat this filtration one more time.
3. Adjust pH to 7.2 with 1 N NaOH or 1 N HCl.
4. Sterilize by filtration through a 0.22-µm-pore-size membrane filter.
5. Dispense 4.5 ml into each tube.
6. Label as LIT medium, with the date of preparation and an expiration date of 1 month.

Hemin Stock Solution

Hemin	100.0 mg
Triethanolamine	10.0 ml
Sterile double-distilled water	10.0 ml

1. Mix triethanolamine with water, add the mixture to a tube containing hemin, shake well, and let dissolve.
2. To make the complete medium, just before inoculation, add 0.5 ml of inactivated fetal bovine serum and 0.25 ml of antibiotic solution. The final concentrations of the antibiotics are 100 U of penicillin per ml, 100 µg of streptomycin per ml, and 0.2 µg of Fungizone per ml.

USAMRU Blood Agar Medium for Leishmaniae (36)

Blood agar base (Difco, B45)	40.0 g
Defibrinated rabbit blood	150.0 ml
Double-distilled water	1,000.0 ml

1. Dissolve 40 g of agar in 1,000 ml of distilled water by heating.
2. Cool the solution, and add 150 ml of defibrinated rabbit blood.
3. Dispense 5-ml portions into 125-by-16-mm screw-cap tubes. Position the tubes at a slant, and allow to cool, preferably on ice, to produce moisture of condensation in the tubes.
4. Incubate the tubes at 35°C for 24 h to ensure sterility. Antibiotics (penicillin and streptomycin) can be added if necessary (see the antibiotic formula in this chapter). The final concentrations of antibiotics are 100 U of penicillin per ml and 100 µg of streptomycin per ml.
5. Inoculate the medium with aspirate material or triturated tissue from biopsy specimen.

Note This medium has been especially useful for primary isolation of the *Leishmania braziliensis* complex in Latin America.

Liquid Media for Cultivation of Hemoflagellates

Hendricks et al. have reported on the highly successful use of liquid culture media for the rapid cultivation of various species of *Leishmania* and *Trypanosoma* (33). Schneider's *Drosophila* medium (GIBCO, Grand Island, NY), supplemented with 30% (vol/vol) fetal calf serum, has been used in making primary isolates from human and animal infections as well as for routine maintenance of a wide variety of leishmanial and trypanosomal species in the laboratory. Both Schneider's medium and Grace's insect tissue culture medium (GIBCO) promote better growth of organisms and are less costly than the widely used blood-agar-based media. In addition, Schneider's medium may be freeze-dried for at least 2 years and reconstituted with distilled water in the field, and it will provide excellent culture results.

Stock Antibiotic Solution (To Be Used with All Media)

Sodium penicillin G	1,000,000 U
Streptomycin sulfate	1,000,000 µg
Fungizone	2,000 µg
Sterile double-distilled water	50.0 ml

1. Mix the components thoroughly. The concentration of the stock solution is:

Penicillin	20,000 U/ml
Streptomycin	20,000 µg/ml
Fungizone	40 µg/ml

2. Dispense 1 ml of the antibiotic mixture into sterile screw-cap vials or sterile cryovials.
3. Label as antibiotic solution with the date of preparation and an expiration date of 1 year.
4. Store at –20°C in cryoboxes.

Axenic Culture

1. Remove tubes containing culture medium (one of NNN, modified NNN, or Tobie's medium and one of Schneider's for *Leishmania*; one of NNN or Tobie's medium and one of LIT for *T. cruzi*) from 4°C, add fetal bovine serum and antibiotics if required, and incubate at 20 to 23°C for 1 to 2 h.
2. Inoculate the specimen (aspirate, scraping, or biopsy material from skin lesions from cutaneous leishmaniasis patients; bone marrow aspirates or splenic aspirates from visceral leishmaniasis patients; or normal skin biopsy specimens, lymph node aspirates, or pieces of liver and spleen from suspected or potential wild- or domestic-animal reservoirs) into the culture tubes. For Chagas' disease, inoculate a few drops of buffy coat into the culture tubes.
3. Add 0.5 ml of overlay (either saline or other overlay, depending on the medium). The organisms will develop in the fluid condensate and overlay at the bottom of the slant (Fig. 8.5).
4. Incubate the tubes at 20 to 24°C.
5. Once every 2 to 3 days, remove a drop of medium and examine it under the low power (100×) of a microscope, preferably one equipped with phase-contrast optics.
6. If promastigotes are seen, inoculate a couple of drops of the medium into fresh culture tubes. Add a couple of drops of 0.85% saline or the overlay solution (depending on the culture medium used) to the old tube.
7. If visible contamination occurs, add antibiotics to the overlay (to contain 200 U of penicillin/ml and 200 µg of streptomycin/ml). The parasites will not proliferate if bacterial contamination is present.
8. Incubate the tubes containing the patient specimen for at least 2 weeks at 28°C or room temperature.
9. If no organisms are seen even after 2 weeks of incubation, examine several drops of fluid under the microscope for promastigotes. The culture should be observed for 1 month before being signed out as negative. Transfers to fresh medium should be made once or twice a week after the culture is established.

Quality Control for Flagellates of Blood and Tissue

The following control strains should be available when using these cultures for clinical specimens: ATCC 30883 (*Leishmania mexicana*) and ATCC 30160 (*T. cruzi*).

1. Check all reagents and media at least once a week. The media should be free of any signs of precipitation and bacterial and/or fungal contamination.
2. The microscope(s) should have been calibrated within the last 12 months, and the original optics used for the calibration should be in place on the microscope(s). The calibration factors for all objectives should be posted on the microscope for easy access.
3. Maintain stock cultures of *Leishmania* spp.
   A. Transfer stock cultures weekly.
      a. Stock organisms should always be cultured at the same time a patient specimen is inoculated into culture medium.
      b. If the stock organisms multiply and remain viable during the 96 h, patient results can be reported.
   B. Stain
      a. Stain a slide prepared from stock culture in parallel with the patient slide.
      b. Staining results are acceptable when the control organisms stain well.

Note Cultivating the organism from suspected materials provides a definitive diagnosis, but it may take as many as 3 to 7 days. Every effort, therefore, must be made to microscopically examine wet smears and/or permanent stained smears so that appropriate therapy can be instituted without delay if findings are positive (Fig. 8.6) (37, 38).

Toxoplasma gondii

The diagnosis of toxoplasmosis may be difficult, because the clinical symptoms mimic a number of various infectious and noninfectious diseases. Serologic tests that are often

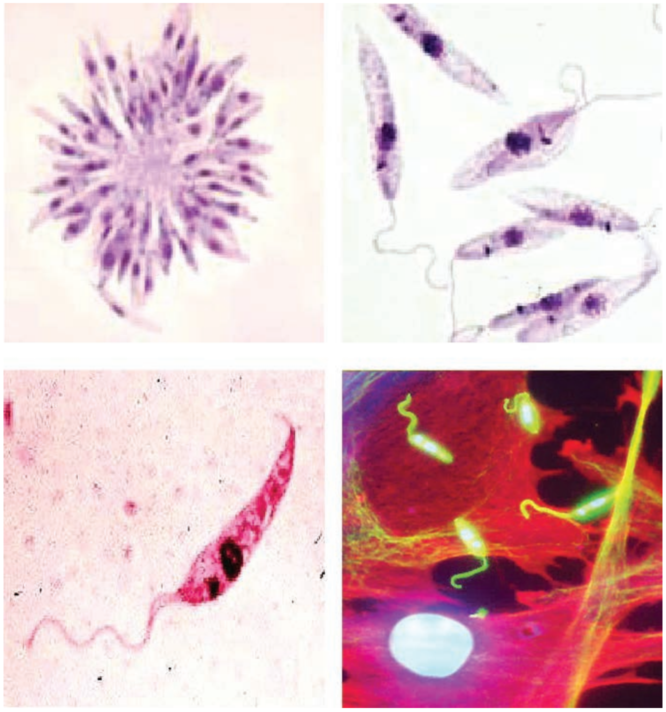


Figure 8.6 *Leishmania* spp. from culture systems. (Upper left) Stained smear of culture fluid sediment [U3] showing promastigotes of *Leishmania* sp. (Upper right) Stained smear of culture fluid sediment showing promastigotes of *Leishmania* sp. (higher magnification). (Lower left) Stained *Leishmania* promastigote. (Lower right) *Leishmania major* promastigotes during infection of primary fibroblast culture. Cells are stained with antitubulin (green) and antiactin (red). (Courtesy of the Pasteur Institute, Molecular Parasitology and Signaling Image Bank).
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used for diagnosis may be insensitive in patients lacking normal immune responses. Sometimes even examination of histologic material does not reveal the organisms. With the increase in the number of laboratories using tissue culture techniques for viral pathogens, these techniques have been used for the isolation and identification of *T. gondii*. The following procedure has been recommended for biopsy specimens, brain, liver, spleen tissue, CSF, amniotic fluid, and buffy coat preparations, and they may be particularly helpful in making the diagnosis in immunosuppressed patients (39). The procedure for buffy coat cells is as follows.

1. Collect 10 ml of blood anticoagulated with preservative-free heparin.
2. Allow the blood to sediment via gravity.
3. Remove the buffy coat (by an aseptic technique), and separate the cells from the plasma by centrifugation at 800 × *g* for 10 min.
4. Wash the buffy coat cells three times with Eagle's minimal essential medium (GIBCO).
5. Inoculate the washed buffy coat material onto complete human foreskin fibroblast (HFF) monolayers (in tubes and shell vials). One HFF tube and two HFF vials should be inoculated for each patient specimen.
6. Observe the cultures weekly for cytopathic effect.
7. The shell vial coverslips can be fixed and stained at 7 and 14 days postinoculation for an indirect fluorescent-antibody (IFA) assay and observed for the tachyzoites.

Note CSF, placental tissue, or other tissues can also be used to inoculate tissue culture monolayers. Uncentrifuged CSF (0.1 to 1 ml) can be used. If more than 1 ml is submitted, the specimen should be centrifuged for 10 min at 500 × *g*. Positive and negative controls must be tested with each set of patient specimen vials.

Three continuous cell lines (HeLa, LLC, and Vero) and three cell culture methods (culture in conventional flasks, culture in membrane-based flasks, and an automated culture system) were investigated (Fig. 8.7). Overall, HeLa was the cell line of choice. Continuous passage in flasks was successful, and HeLa-derived tachyzoites can be used for the dye test, if applicable in your laboratory setting (40). Another study indicates that THP1 cells serve as a good model of invasion for *T. gondii* (41).

Plasmodium and *Babesia* spp.

Techniques for the culture of *Plasmodium falciparum* were described in 1976 (42) and have been improved and modified since that time (43, 44). Life cycle stages of the five *Plasmodium* spp. that infect humans have been established *in vitro*. Of these five, *P. falciparum* and *P. knowlesi* are the only species for which all stages have been cultured *in vitro*. The life cycle includes the exoerythrocytic stage (within liver cells), the erythrocytic stage (within erythrocytes or precursor reticulocytes), and the sporogonic stage (within the vector). Culture media generally consist of a basic tissue culture medium to which serum and erythrocytes are added. Most of the culture methods have been directed toward the stage found in the erythrocyte. This stage has been cultivated in petri dishes or other containers in a candle jar to generate elevated CO_2 levels or in a more controlled CO_2 atmosphere. Later developments employed continuous-flow systems to reduce the labor-intensive requirement for replenishing the system with fresh media. The exoerythrocytic and sporogonic life cycle stages have also been cultivated *in vitro*. Although cultivation is of great help in understanding the biology of *Plasmodium*, it does not lend itself to use for routine diagnostic purposes (7).

The availability of the microaerophilous stationary phase (MASP) culture technique, in which the parasites pro-

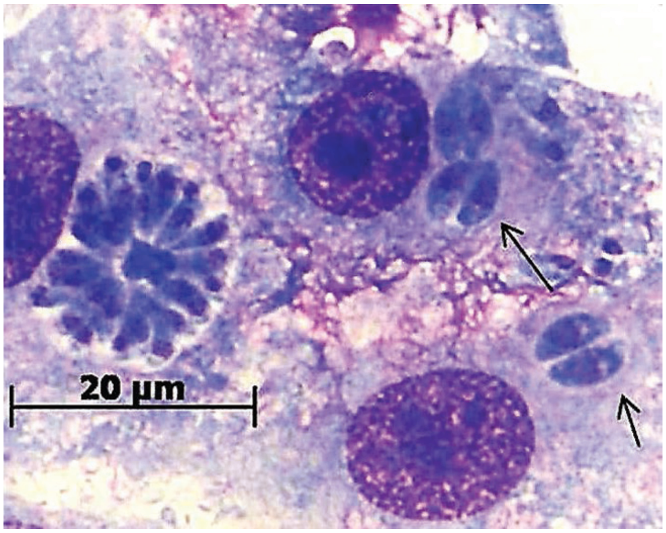

Figure 8.7 *Toxoplasma gondii* RH tachyzoites replicated in Vero cell cultures. A rosette of many tachyzoites is seen at the left and several parasite pairs are at right (arrows). (Courtesy of I. Canedo-Solares, Abstract 42.009, 15th International Congress on Infectious Diseases, Bangkok, Thailand, 2012).
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liferate in a settled layer of blood cells, has provided an opportunity to study *Babesia*, a formerly obscure disease agent regarded as within the purview of veterinary parasitology, in the laboratory. A number of *Babesia* spp. have been established in continuous culture using the MASP technique. It is possible to study the basic biology of the organism—as well as host-microbe interactions, immune factors triggered by the parasite, factors involved in innate resistance of young animals to infection, and antimicrobial susceptibility—to a degree not possible before the availability of cultures. These culture systems can produce quantities of parasite nucleic acid needed for defining phylogenetic relationships, developing diagnostic methods for parasite detection in asymptomatic individuals, and producing parasite antigens and attenuated strains of *Babesia* that could be used for immunization (5).

Cryptosporidium spp.

The *in vitro* cultivation of *Cryptosporidium* has improved significantly in recent years. These obligate intracellular parasites colonize the epithelium of the digestive and respiratory tracts, are often difficult to obtain in significant numbers, produce durable oocysts that defy conventional chemical disinfection methods, and are persistently infectious when stored at refrigerator temperatures (4 to 8°C). While continuous culture and oocyst production have not yet been achieved *in vitro*, routine methods for parasite preparation and cell culture infection and assays for parasite life cycle development have been established. Parasite yields tend to be limited, but *in vitro* growth is sufficient to support a variety of research studies, including assessing potential drug therapies, evaluating oocyst disinfection methods, and characterizing life cycle stage development and differentiation (1). Recent studies indicate that primary human intestinal epithelial cells (PECs) support *Cryptosporidium* better than other existing cell lines (45).

Microsporidia

Although various microsporidia that infect humans can be identified from clinical specimens by serologic and/or molecular methods, none of these methods are commercially available. Unfortunately, in some cases microscopic examination of biopsy specimens does not yield conclusive results. It is also possible that microsporidial organisms may be present in very small numbers, which can be easily missed during routine histologic examinations. Some microsporidia such as *Encephalitozoon*, *Enterocytozoon*, *Vittaforma*, *Trachipleistophora*, and *Anncaliia* spp., even when they are present in small numbers, can become established in cell cultures, thus facilitating their identification at a later time. Therefore, attempts at culturing these organisms should be made whenever possible, since many clinical laboratory personnel are familiar with cell culture methodology (11). *Anncaliia algerae* has been successfully grown on the rabbit kidney cell line, RH-13 (46). Cell lines from goldfish skin (GFSK-S1) and brain (GFB3C-W1) and ZEB2J from zebrafish embryos, FHMT-W1 from fathead minnow testis, and Sf9 from ovaries of a fall armyworm moth were also found to be successful. All cultures were maintained at 27°C. Infection was judged to have taken place by the appearance of sporonts and/or spores in cells and occurred in all cell lines. These results suggest that cells of a wide range of vertebrates support *A. algerae* growth *in vitro* and fish cells can produce spores infectious to cells of mammals, fish, and insects (46). Expanded information on cell lines, media and supplements used for the culture of microsporidia causing human infections can be seen in Table 8.2 and Fig. 8.8 and 8.9.

Animal Inoculation

Most routine clinical laboratories do not have the animal care facilities necessary to provide animal inoculation capabilities for the diagnosis of parasitic infections. Host specificity for many animal parasite species is a well-known fact which limits the types of animals available for these procedures. In certain suspect infections, animal inoculation may be requested and can be very helpful in making

TABLE 8.2 Cell lines, media, and supplements that have been used to cultivate microsporidia causing human infections (adapted from references 11 and 46)

Species	Cell lines[a]	Medium	Supplements and culture conditions[b]
Encephalitozoon cuniculi	E6, HLF, MDCK, MRC-5, Vero, HeLa	EMEM + 2 mM glutamine	5 or 10% FBS or FCS and antibiotics (penicillin and streptomycin or gentamicin and amphotericin B), with or without 5% CO_2
Encephalitozoon hellem	MDCK, E6, HLF, MRC-5, RK-13, FBF, Vero	EMEM + 2 mM glutamine, RPMI	5 or 10% FBS or FCS and antibiotics (penicillin and streptomycin or gentamicin, amphotericin B), with or without 5% CO_2
Encephalitozoon intestinalis	E6, HLF, MDCK, HEL, MDM, RK-13, I047, HT-29, Caco-2, Vero	EMEM + 2 mM glutamine, RPMI 1640, DMEM + 2 mM glutamine	5 or 10% FBS, 5 or 10% FBS or FCS, antibiotics (penicillin and streptomycin; or gentamicin; or a cocktail of amoxicillin, vancomycin, gentamicin, and flucytosine; or amphotericin B), with or without 5% CO_2
Enterocytozoon bieneusi	E6, HLF, HT-29, Caco-2, MDCK, RK-13	EMEM + 2 mM glutamine	5 or 10% FBS, 5% or 10% FBS, EGF, transferrin, insulin, HC, selenium, HEPES
Vittaforma corneae	SIRC, Vero, MDCK, E6, HLF, MRC-5	EMEM + 2 mM glutamine	5% or 10% FBS, gentamicin, with or without 5% CO_2
Trachipleistophora hominis	MDCK, RK-13, COS-1, L6-C10, MM	DMEM + 2 mM glutamine	10% FBS, 10% HS, gentamicin, with or without 5% CO_2
Anncaliia algerae (formerly *Brachiola algerae*)	E6, HLF, RK-13, GFSK-S1, GFB3C-W1, ZEB2J, FHMT-W1, Sf9	EMEM + 2 mM glutamine	5 or 10% FBS, gentamicin and amphotericin B, without 5% CO_2

[a] E6, monkey kidney; FHMT-W1, fathead minnow testis; GFSK-S1, goldfish skin; GFB3C-W1, goldfish brain; HLF, human lung fibroblasts; MDCK, Madin-Darby canine kidney; MRC-5, lung fibroblasts; RK-13, rabbit kidney; FBF, fetal bovine lung fibroblasts; HEL, human embryonic lung; HeLa, cervix carcinoma cell; MDM, monocytes; I 047, intestinal; HT-29, human adenocarcinoma; Caco-2, human colon carcinoma; Sf9, ovaries of fall armyworm moth; SIRC, rabbit corneal epithelium; COS-1, green monkey kidney; L6-C10, human cell line; MM, mouse myoblasts; Vero, African green monkey kidney; ZEB2J, zebrafish embryos.

[b] FBS, fetal bovine serum; FCS, fetal calf serum; EGF, epidermal growth factor; HC, hydrocortisone; HS, human serum.

Figure 8.8 (A) An E6 cell infected with *E. intestinalis*. Magnification, ×1,200. Note the well-defined multiple parasitophorous vacuoles (PV); N, host cell nucleus. (B) An HLF cell culture completely destroyed by *E. hellem*. Note the spores with everted polar tubules (at arrows), Magnification, ×600. All three species of *Encephalitozoon* destroy the cell culture, and often the cell cultures are completely covered by spores that either are intact or have discharged their polar tubules. Note It is very unusual to see the extruded polar tubules in routine clinical specimens such as urine or stool. (Courtesy of Govinda Visvesvara, from Visvesvara G, *Clin Microbiol Rev* **15:**401–413, 2002).
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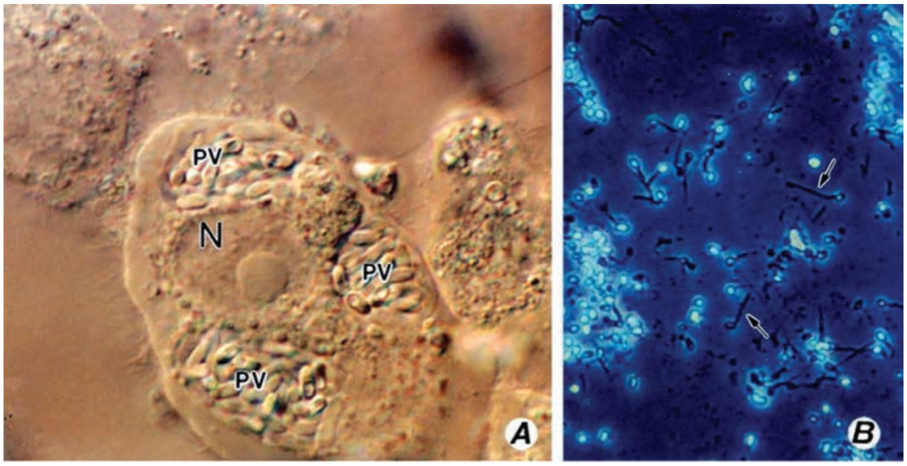

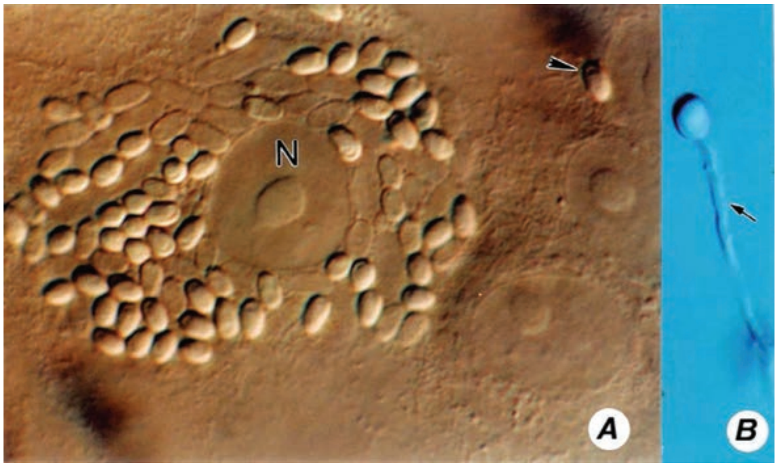

Figure 8.9 (A) A host cell infected with *Anncaliia* (*Brachiola*) *algerae*. Note the arrangement of spores around the host (E6 cell) nucleus (N). A single spore is probably in the process of infecting an adjacent cell (arrowhead). Magnification, ×1,200. (B) A spore with an everted polar tubule. Magnification, ×1,200. (Courtesy of Govinda Visvesvara, from Visvesvara G, *Clin Microbiol Rev* **15**:401–413, 2002).
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the diagnosis, although animal inoculation certainly does not take the place of other, more routine procedures.

Leishmania spp.

The hamster is the laboratory animal of choice for the isolation of any form of *Leishmania* spp. A generalized infection results after intraperitoneal inoculation; spleen impression smears should be examined for the presence of organisms.

1. Aspirates or biopsy material obtained under sterile conditions from cutaneous ulcers, lymph nodes, spleen, liver, bone marrow, buffy coat cells, or CSF may be used for inoculation.
2. Material (0.25 to 1 ml) obtained from these sources should be inoculated under sterile conditions by the intraperitoneal route. Material from patients with mucocutaneous leishmaniasis should be inoculated by the intranasal route or into the feet.
3. Young (either sex) hamsters (2 to 4 months old) should be used for this procedure.
4. If the animal dies several days after inoculation, splenic aspirates should be examined for the presence of organisms. The material should be prepared as thick blood films and stained with Giemsa or other blood stains.

Note The infection develops slowly in hamsters; several months may be required to produce a detectable infection. For this reason, culture procedures are usually selected as more rapid means of parasite recovery. Intranasal lesions or those in the feet may develop very slowly; experimental animals should be kept for 9 to 12 months before a negative report is sent out.

Trypanosoma spp. (15)

Several laboratory animals can be used for the recovery of trypanosomes. Organisms are usually found in the blood of the animals within the first week after inoculation; however, if no organisms are found, the animals should be checked at the end of 2 and 4 weeks before results are reported as negative. *Trypanosomes are infectious, so extreme care should be used when examining any blood or tissue suspected to contain these organisms.*

1. Blood, lymph node aspirates, tissue, or CSF obtained under sterile conditions may be used for inoculation.
2. Material (up to 2 ml) should be inoculated intraperitoneally into guinea pigs or white rats for *Trypanosoma gambiense* and *T. rhodesiense* and into white mice for *T. cruzi*.
3. The number of organisms in the blood may vary; therefore, smears should be prepared frequently (every few days) over a 4-week period after inoculation.

Note Rats should be checked for the presence of *T. lewisi* (common parasite in rats) before inoculation to prevent a possible false-positive result.

Toxoplasma gondii

All common laboratory animals can be infected by *T. gondii*. White rats and mice are generally used. White rats develop a chronic infection that can be a useful means of maintaining a strain of organisms. Mice that have been inoculated by the intraperitoneal route develop a fulminating infection that leads to death within a few days. Tremendous numbers of organisms can be recovered from the ascitic fluid. These specimens should be handled carefully to avoid an accidental laboratory infection.

General Procedure

1. The *Toxoplasma* organisms are found throughout the body after dissemination via the bloodstream. Any body tissue or fluid can be used for animal inoculation; the most common specimens are blood, lymph node fluid, and CSF.

2. The material used for inoculation (the amount may be very small [less than 0.25 ml]) should be obtained under sterile conditions and should be inoculated via the intraperitoneal route.
3. White mice of either sex and any age can be used. The animals should be checked daily for symptoms of illness.
4. After several days, the organisms can be recovered at necropsy from the peritoneal fluid of the mouse. The material should be prepared as thick blood films and stained with Giemsa or other blood stains.
5. Blind passage of peritoneal fluid to additional mice is recommended if the original mice appear to be negative.
6. If mice survive for up to 6 months, serum can be tested for the presence of antibody.

Procedure for Tissue

1. Grind the tissue in sterile 0.85% NaCl.
2. Prepare a 10% suspension (10% tissue, 90% NaCl).
3. Inoculate six mice via the intraperitoneal route. Each mouse should receive 1 ml.
4. The mice can be reinoculated the following day with an additional 1-ml dose.
5. Check the mice daily for symptoms.
6. If the animals are not sick at the end of 6 days, examine 1 drop of peritoneal fluid from each mouse directly and then stain it with Giemsa stain.

Figure 8.10 Illustration of the process of xenodiagnosis used for the diagnosis of Chagas' disease. (Illustration by Sharon Belkin.)

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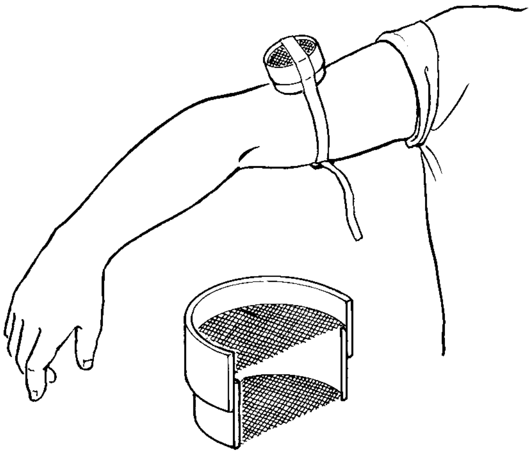

Xenodiagnosis

Xenodiagnosis is a technique that uses the arthropod host as an indicator of infection. Uninfected reduviid bugs are allowed to feed on the blood of a patient who is suspected of having Chagas' disease (*T. cruzi* infection) (47) (Fig. 8.10). After 30 to 60 days, feces from the bugs are examined over a 3-month time frame for the presence of developmental stages of the parasite, which are found in the hindgut of the vector. This type of procedure is used primarily in South America for field work, and the appropriate bugs are raised in various laboratories specifically for this purpose.

The term "xenodiagnosis" has also been applied to the diagnosis of trichinosis (*Trichinella spiralis*). Muscle tissue from a patient suspected of having the disease is fed to uninfected rats; the rats are then checked after the appropriate time for the presence of *T. spiralis* larvae, particularly in the diaphragm. This procedure is rarely requested and is not available in most clinical laboratories.

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9

Fixation and Special Preparation of Fecal Parasite Specimens and Arthropods

Fixation of parasite specimens and arthropods
- Protozoa
- Solutions to induce relaxation in adult helminths
- Nematodes
- Trematodes
- Cestodes
- Helminth eggs and larvae
- Arthropods

Mounting and staining of parasite specimens for examination
- Nematodes
- Trematodes
- Cestodes

Mounting of arthropods for examination
- Mites
- Fleas and lice
- Ticks
- Miscellaneous arthropods

Fixation of Parasite Specimens and Arthropods

Adequate fixation of parasites is important not only for diagnostic procedures but also as a means of preserving positive material for personnel and student training. There are many fixatives and preservatives available; however, only the more common ones are presented here.

Although this chapter does not include commentary related to the histopathology laboratory, some information about formalin fixatives may be helpful. Concerns about the toxicity of formalin, particularly in the quantities used in a routine histology laboratory, have led to trials of alternative methods of tissue fixation that do not require formalin. Alcohol-based fixatives have been proposed as optimal for immunohistochemical and nucleic acid methods and may be useful for diagnostic light microscopy. Some laboratories have converted to use of an alcoholic fixative (containing 56% ethanol and 20% polyethylene glycol). Although comparative scores between the two were slightly in favor of the formalin, there were no significant differences in terms of tissue architecture, cell borders, cytoplasm, nuclear contours, chromatin texture, red blood cell membranes, and uniformity of staining. Alcohol-polyethylene glycol appears to be a satisfactory alternative to formalin in routine diagnostic surgical pathology (1). In another study, Histochoice produced a staining intensity that was comparable, and in many cases superior, to that of formalin (2).

Formalin-fixed and paraffin-embedded tissues present particular challenges for proteomic analysis. However, most archived tissues in pathology departments and tissue banks worldwide are available in this form. Different approaches to removal of the embedding medium and protein digestion have been developed, thus releasing tryptic peptides, which are suitable for analysis by liquid chromatography-mass spectrometry. Peptide identifications made using this approach are comparable to those from matched fresh frozen tissue. Apparently, a high level of sequence coverage can be seen for proteins under study (3).

The effect of fixation on the degradation of nuclear and mitochondrial DNA in different tissues has been examined (4). Samples of different tissues were preserved in seven fixatives for periods extending from 1 to 336 days to determine which fixatives reduce the time-dependent degradation of DNA and preserve the histologic structure. For long-term storage in combination with amplification

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of nuclear and mitochondrial DNA, consistent results were obtained with Carnoy's solution and glutaraldehyde. Variable results were observed for buffered formalin. In regard to comparison of the different tissues, the quantities recovered from skeletal muscles and kidneys were larger than from other tissues. These fixative studies will become even more important as molecular methods applied to fixed tissues become more common.

Molecular characterization of morphologic change requires precise tissue morphology and RNA preservation; however, traditional fixatives usually result in fragmented RNA. To optimize molecular analyses of fixed tissues, morphologic and RNA integrity in rat liver was assessed when sections were fixed in 70% neutral buffered formalin, modified Davidson's II medium, 70% ethanol, UNIFIX, modified Carnoy's solution, modified methacarn, Bouin's medium, phosphate-buffered saline, or 30% sucrose. Each sample was treated with standard or microwave fixation and standard or microwave processing, and sections were evaluated microscopically. RNA was extracted and assessed for preservation of quality and quantity (5). Modified methacarn, 70% ethanol, and modified Carnoy's solution resulted in tissue morphology providing a reasonable alternative to formalin. Modified methacarn and UNIFIX, best preserved RNA quality. Neither microwave fixation nor processing affected RNA integrity relative to standard methods, although morphology was somewhat improved. Modified methacarn, 70% ethanol, and modified Carnoy's solution provided acceptable tissue morphology and RNA quality with both standard and microwave fixation and processing methods. Of these three fixatives, modified methacarn provided the best results and can be considered a fixative of choice where tissue morphology and RNA integrity are being assessed in the same specimens. In another study, the PAXgene Tissue System preserves histology similarly to formalin, but does not chemically modify RNA. RNA purified from PAXgene fixed tissues performs as well as RNA from fresh frozen tissue in real-time PCR regardless of amplicon length (6).

PCR has also been used to identify tissue-embedded ascarid nematode larvae. Two sequences of the internal transcribed spacer (ITS) regions of the rRNA gene of the ascarid parasites (ITS1 and ITS2) were compared with those registered in GenBank. PCR amplification of the ITS regions was sensitive enough to detect a single larva of *Ascaris suum* mixed with porcine liver tissue. These results suggest that even a single larva embedded in tissues from patients with larva migrans could be identified by sequencing the ITS regions (7).

Although formalin fixation is the most common storage, transportation, and preservation method for stool samples, it dramatically reduces the ability to extract DNA from stool samples for PCR-based diagnostic tests. Apparently, the deleterious effects of formalin are both time and concentration dependent and may result from fragmentation of fixed DNA during its purification. This has been seen in studies of the effect of formalin fixation on PCR of *Entamoeba histolytica* (8). The TOTAL-FIX stool collection kit is a single-vial system that provides a standardized method for untrained personnel to properly collect and preserve stool specimens for the detection of helminth larvae and eggs, protozoan trophozoites and cysts, coccidian oocysts, and microsporidian spores. Concentrations, permanent stains, most fecal immunoassays, and some molecular methods can be performed from a TOTAL-FIX preserved specimen. TOTAL-FIX is a mercury-, formalin-, and PVA-free fixative that preserves parasite morphology and helps with disposal and monitoring problems encountered by laboratories (www.med-chem.com; accessed 7/24/13). TOTAL-FIX is similar to UNIFIX and Zinc PVA (Z-PVA), commonly used fixatives that have been commercially available and used in many laboratories since 1992 (9, 10).

Parasites found in feces can be preserved in one of two ways (9, 10). One procedure involves thorough mixing of the fecal specimen directly in the fixative. This mixture can then be stored at room temperature without any additional processing. There are several disadvantages of this method: (i) if mixing is not complete, some of the organisms will not be well preserved and will disintegrate during storage; (ii) if the number of organisms is small, a random aliquot may not reveal the parasites; and (iii) the proper ratio of fixative to specimen may not be correct when one is working with larger volumes of material.

The second approach is to clean and separate the organisms from excess debris and concentrate them before fixation. The specimens can be mixed with a large volume of water, passed through a series of screens (large to small pore size), and finally allowed to sediment in a conical container (pilsner glass). After approximately 1 h, the sediment can be washed several times, resedimented, and finally fixed with hot (60 to 63°C) fixative to quickly preserve and stop any further development of certain helminth (*Ascaris* and *Trichuris*) eggs. The main disadvantage of this method is extensive washing and manipulation, which may lead to some organism distortion or destruction before fixation. Also, this approach is not very practical unless specimens are being processed for teaching/holding purposes.

The adult forms of most helminths must be relaxed prior to fixation. If this preliminary step is not performed, the worms often contract and curl up when they come in contact with the fixative, thus preventing visual examination of some of the key morphologic characteristics. Several methods, including those involving tap water, physiologic saline, and dilute menthol, are available for this purpose.

Protozoa

Definitive morphology needed for identification of protozoan trophozoites and cysts is best seen on the permanent stained smear, which can be prepared from fresh fecal specimens or from stool that has been submitted to the laboratory in fixative. No washing techniques should be used before bulk fixation, because the trophozoites will be destroyed. Immediately after collection or submission to the laboratory, the specimens should be thoroughly mixed with the fixative of choice. The ratio of fixative to stool should be at least 3 parts fixative to 1 part stool, and the fixation time should be at least 4 h (or less, depending on the amount of specimen used). The normal collection vial systems usually require a fixation time of at least 30 min. If an entire fecal specimen is used, 4 h is recommended. Individual slides can then be prepared from bulk-fixed material by a technique described by Scholten and Yang (11). This method involves centrifugation and smear preparation from the sediment. Although formalin-preserved specimens (except for sodium acetate-acetic acid-formalin-preserved fecal material) are not recommended for the preparation of permanent stained smears, formalin fixation for cyst preservation is recommended for the preservation of teaching specimens that will be examined as wet mounts only. The morphology of cysts can often be seen in formalinized wet mounts sufficiently well to identify organisms to the species level or certainly to be highly suggestive.

Streck tissue fixative has also been tested as a substitute for formalin and polyvinyl alcohol in fecal preservation. Stool samples were examined microscopically as follows: (i) in wet mounts (by bright-field and epifluorescence microscopy), (ii) in modified acid-fast-, trichrome-, and safranin-stained smears, and (iii) with two commercial test kits. Specific results showed that *Cyclospora* oocysts retained full fluorescence, modified acid-fast- and safranin-stained smears of *Cryptosporidium* and *Cyclospora* oocysts were equal in staining quality, and results were comparable in the immunofluorescence assay and enzyme immunoassay commercial kits. However, stool fixed in Streck tissue fixative and stained with trichrome showed unacceptable staining quality compared with stool fixed in preservatives, many of which contained PVA (fecal adhesive). Thus, Streck fixative is an excellent substitute for formalin; however, modifications to the trichrome procedure will be required to improve the staining characteristics of protozoan parasites (12). With the introduction of the Universal Fixative, TOTAL-FIX, formalin is no longer a consideration.

Formalin-Saline Solution

Although formalin is generally used at room temperature for routine diagnostic work, hot (60 to 63°C) 5% formalin in a ratio of 3 parts fixative to 1 part stool is recommended for bulk specimens containing intestinal protozoa. Long-term storage of protozoa is enhanced with buffered formalin; *the solution should be replaced every 6 months*. Acceptable morphology for teaching purposes can be maintained for 6 months to several years, depending on the organism and the lag time between specimen collection and fixation. The organism cytoplasm will tend to become glassy or granular with very poor nuclear definition. Cysts of *Entamoeba coli* and *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) tend to maintain their morphologic characteristics for years, whereas cysts of *E. histolytica* and *E. hartmanni* do not preserve well (Fig. 9.1). All of these cysts retain their ability to take up iodine in a wet, direct smear.

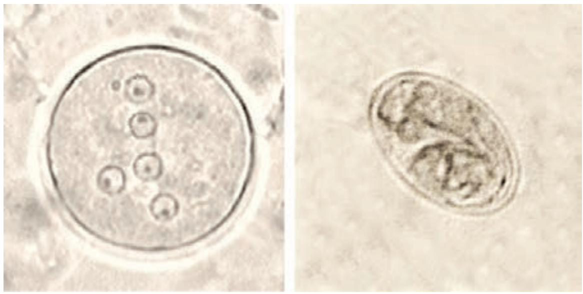


Figure 9.1 (Left) *Entamoeba coli* cyst (unstained). (Right) *Giardia lamblia* cyst (unstained). doi:10.1128/9781555819002.ch9.f1

The standard 10% formalin will fix protozoan cysts; however, it does not preserve morphology as well as does the buffered 5% formalin.

Formalin-Saline Solution

Formaldehyde	5 ml
0.85% saline solution	95 ml

Formaldehyde is normally purchased as a 37% HCHO solution; however, for dilution, it should be considered to be 100%.

Buffered Formalin-Saline Solution

Formaldehyde	400 ml
0.85% saline solution	7,600 ml
Na_2HPO_4	6.10 g
NaH_2PO_4	0.15 g

10% Formalin Solution

Formaldehyde	10 ml
Distilled water	90 ml

Solutions To Induce Relaxation in Adult Helminths (13)

Tap Water or Physiologic Saline

1. Place living worms into a dish containing tap water or 0.85% NaCl solution.
2. Refrigerate the dish for 2 to 4 h.

Note Tap water generally works better than saline; also, the trematodes expel their eggs, thus allowing the internal morphology to be seen more clearly.

Dilute Menthol (14)

1. Dissolve 24 g of menthol crystals in 10 ml of 95% ethyl alcohol, and mix well.
2. Store the solution until needed.
3. Place living worms into a dish containing 100 ml of tap water or 0.85% NaCl.
4. Add 1 drop of menthol solution to the dish containing the worms.
5. Refrigerate the dish for 2 to 3 h.

Note The use of menthol accelerates relaxation of the worms. When this approach is used, the rostellum on the scolex of adult cestodes remains extruded during fixation.

Nematodes

Roundworms can be preserved with several different fixatives (Fig. 9.2). Formalin is usually not recommended, since it tends to harden the tissues. Nematodes can be killed with hot water (60 to 63°C) and then transferred to a preservative, such as alcohol-glycerin or alcohol-formalin-acetic acid (AFA). It is recommended that nematode larval stages be fixed in hot water. Direct fixation in preservatives may cause the cuticle to become "sticky," and the larvae will be damaged when they adhere to the glass container (15).

Alcohol-Glycerin

Alcohol (70%) containing 5% glycerin is an excellent fixative for most nematodes and should be used hot (60 to 63°C). Specimens can be left in this original fixing solution indefinitely. The glycerin will protect the specimen if the alcohol evaporates. Fixative evaporation is another reason why fixed specimens should be routinely checked every 6 months for possible fixative replacement.

Alcohol-Glycerin

95% ethyl alcohol	70 ml
Distilled water	25 ml
Glycerin	5 ml

AFA Solution

A solution of alcohol, formalin, and acetic acid can be routinely used for nematodes, trematodes, and cestodes. The fixative should be used hot (60 to 63°C); after fixation for 24 h, parasites can be stored in the alcohol-glycerin mixture.

AFA Solution

Formaldehyde	10 ml
95% Ethyl alcohol	50 ml
Acetic acid, glacial	5 ml
Distilled water	45 ml

Glacial Acetic Acid

Undiluted glacial acetic acid is recommended for fixation of the smaller nematodes (Fig. 9.3). They are killed instantly in an extended position. This fixative is not recommended for the larger worms, such as *Ascaris lumbricoides*. The acetic acid will clear the worm tissue so that the internal structure becomes visible. Morphologic characteristics can be seen under the microscope if the worm is placed in water on a slide. Worms fixed with acetic acid can be placed in AFA for 24 h and then into alcohol-glycerin for long-term storage.

Figure 9.2 Adult *Ascaris lumbricoides* nematode (male worm). doi:10.1128/9781555819002.ch9.f2

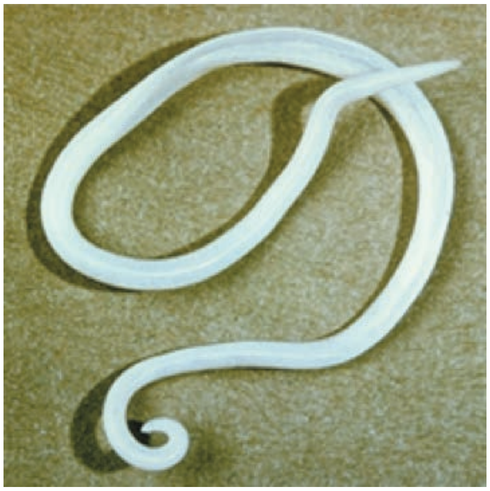
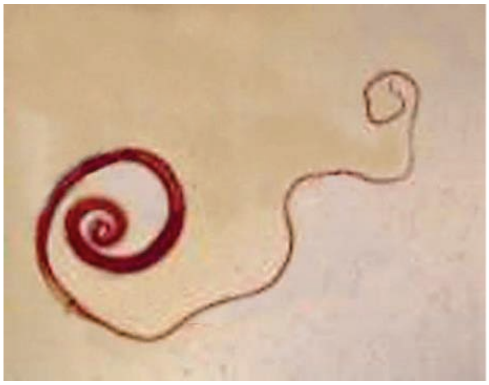


Figure 9.3 Adult *Trichuris trichiura* nematode (note the "whip-like" appearance; this is a much smaller adult worm than *Ascaris lumbricoides*). The small end attaches to the intestinal wall, while the large end is free in the bowel lumen. doi:10.1128/9781555819002.ch9.f3



Dilute Formalin

Although formalin is not recommended as a general fixative for nematodes, a dilute solution (1 to 2%) can be used to kill *A. lumbricoides*. Higher concentrations should not be used since the differences in osmotic pressure may cause the worms to rupture. After storage in dilute formalin for at least 24 h, the worms can be transferred to 10% formalin for long-term storage.

Note *Ascaris*, *Toxocara*, and *Baylisascaris* eggs continue to develop in formalin (10% or less) to the infective stage and remain viable and infective for a number of months (Fig. 9.4). Worms should be handled with caution, particularly if they are to be dissected.

Trematodes

Most trematodes are very muscular and may contract when placed in fixatives. Living specimens should be placed in cold 0.85% saline for 30 min to several hours (depending on the size of the worm) before fixation. Specimens can be placed on a slide in a petri dish and then covered with another slide or coverglass to flatten the worm. Do not apply pressure, since doing so may distort internal organs (Fig. 9.5).

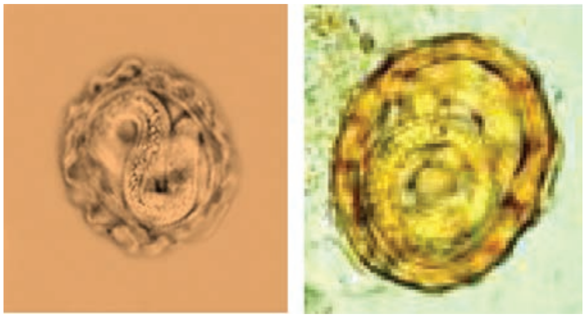
Note Formalin is never recommended for the fixation of trematodes (16).

AFA fixative (see above) should be used hot (60 to 63°C); the worms can be stored in AFA or transferred to 70% alcohol for long-term storage.

Cestodes

Acid fixatives dissolve the characteristic calcareous corpuscles found in tapeworm tissue and should not be used. Since these corpuscles may be used to diagnose tapeworm tissue in histologic sections, buffered formalin or non-acid-containing fixatives are recommended (16).

Figure 9.4 *Ascaris lumbricoides* fertilized eggs. Note the fully developed larva within each egg; these eggs were viable when photographed. In some cases, the moving larva can be seen, even in formalin-preserved specimens.
doi:10.1128/9781555819002.ch9.f4



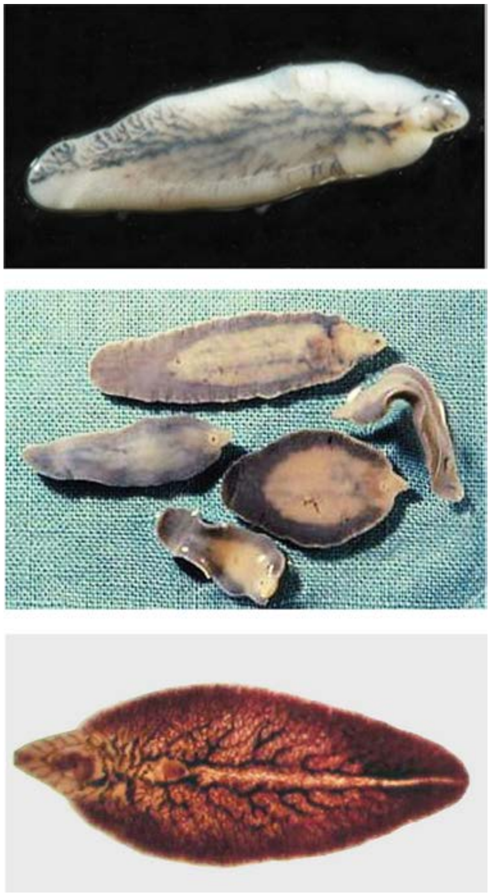


Figure 9.5 Adult *Fasciola hepatica* trematodes. (Upper) Fresh trematode (unfixed, unstained). (Middle) Unstained flukes. (Bottom) Stained fluke (note the morphologic details that can be seen after staining).
doi:10.1128/9781555819002.ch9.f5

Formalin

Hot (60 to 63°C) buffered formalin is recommended for the fixation of cestodes. If the whole worms are immediately swirled around in the container of fixative, they will be rapidly fixed with minimal contraction of the proglottids. If placed in cold formalin, the proglottids tend to contract; subsequent India ink injection will be more difficult to accomplish.

AFA Solution

AFA fixes tapeworm tissue well; however, the acid dissolves the calcareous corpuscles. The worms can be transferred to 70% alcohol for long-term storage (Fig. 9.6).

Note All tapeworms and proglottids should be handled with care, especially if the species has not been determined. The eggs of *Taenia solium* (cysticercosis) and *Hymenolepis nana* are infectious for humans (Fig. 9.7).

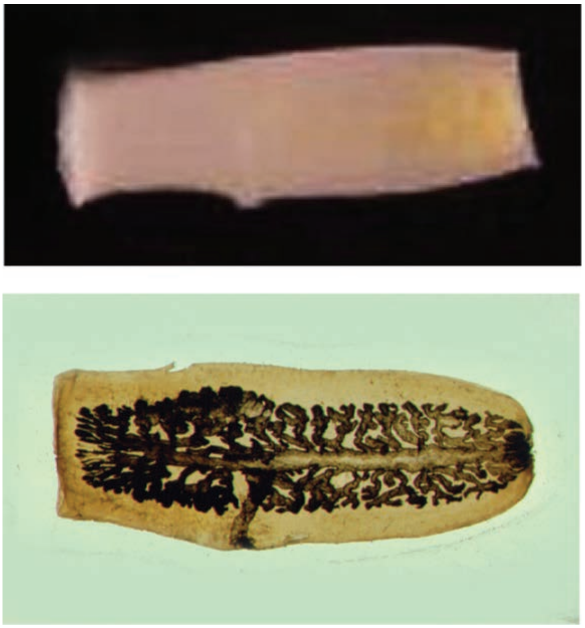
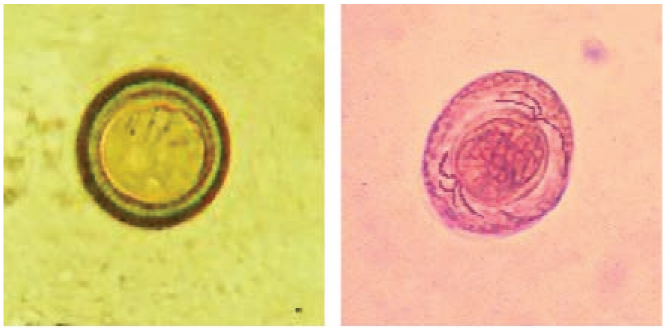


Figure 9.6 *Taenia saginata* proglottids. (Upper) Note that morphologic details cannot be seen in this preserved but unstained preparation. (Lower) After India ink injection, the uterine branches are now visible and can be counted. doi:10.1128/9781555819002.ch9.f6

Helminth Eggs and Larvae

Whole fecal specimens can be mixed directly with an appropriate fixative, although it is recommended that the organisms be concentrated before fixation. The use of formalin-saline as described for the fixation of protozoa is recommended for helminth eggs and larvae. If well preserved, most helminth eggs maintain their morphologic characteristics indefinitely.

Figure 9.7 (Left) *Taenia* spp. egg (note the striated shell and hooklets seen within the embryo (oncosphere). (Right) *Hymenolepis nana* egg (note the six-hooked embryo and the polar filaments that are found between the oncosphere and the shell). doi:10.1128/9781555819002.ch9.f7



Arthropods

Clinical laboratory personnel may receive arthropods for identification from various patient sources (surface of the body, stool, sputum, etc.) (16, 17). Specimens may be submitted when the actual source is unknown (implicated in a bite, found in the house or yard, etc.). Small, wingless insects and other arthropods should be placed in alcohol (70 to 95%), where they can remain indefinitely. Formalin fixative is not recommended for such specimens. Most flying insects should be killed in a chloroform tube or cyanide bottle and then preserved as a dry mount. Large maggots and other larvae can be killed in hot water first to prevent body shrinkage and contraction when placed in alcohol (18). A number of books also contain detailed information on the collection, preservation, and mounting of arthropods (19–21).

DNA sequencing has become much more common in surveys of archival research collections, particularly in reviewing rare taxa of arthropods. As an example, marine invertebrates have historically been maintained in ethanol following initial fixation in formalin. These collections often represent rare or extinct species or populations, provide detailed time series samples, or come from presently inaccessible or difficult-to-sample localities. Results obtained from preserved crustaceans in archival research collections indicate that in the absence of fresh or frozen tissues, archived formalin-fixed, ethanol-preserved specimens will prove a useful source of material for gene sequence data analysis by PCR and direct sequencing (22). It has also been determined that the now widespread use of critical-point drying of wasps and other insects from alcohol is advocated as a potential source of DNA from rare taxa (23).

Another problem that has been documented involves morphology changes seen with the use of various fixatives. Thus, fixation and mounting can significantly influence the morphometric analysis of mites and other arthropods. It is recommended that morphometric studies be conducted using consistent methods to reduce experimental bias and that the methods used be reported in publications dealing with morphometric analyses (24).

Modified Berelese's medium is an all-purpose medium that kills, fixes, and preserves many arthropod specimens. No dehydration in alcohol is necessary.

Modified Berelese's Medium

Gum acacia	8 g
Distilled water	8 ml
Glycerin	5 ml
Chloral hydrate	70 g
Acetic acid, glacial	3 ml

Mounting and Staining of Parasite Specimens for Examination

Following preservation, many staining and mounting techniques for the preparation of permanent or semipermanent mounts of helminth eggs and larvae and of arthropods may be used.

Nematodes

Because the cuticle of nematodes prevents the uptake of stain, these worms are usually rendered transparent with glycerin or lactophenol. In this way, the internal morphology can be observed for identification purposes. Specific morphologic features that can be seen include the cuticle, alimentary tract, and reproductive structures. Small nematodes can be mounted in glycerin jelly. Standard mounting media containing resins and dehydrating agents are generally not satisfactory because the worms tend to collapse and become distorted during dehydration.

Glycerin Jelly Preparation

Specimens are transferred from pure glycerin directly into the glycerin jelly, a medium that will gel at room temperature, thus providing a semipermanent mount.

Glycerin Jelly (Refractive Index, 1.47)

Gelatin, granulated	10 g
Distilled water	60 ml
Glycerin, pure	70 ml
Phenol crystals, melted	0.5 ml

Dissolve the gelatin in water in a beaker in a water bath. Add the glycerin and melted phenol. Store in small, wide-mouth bottles, and refrigerate. Remelt in a hot water bath before use. Dispense with a dropper onto the slide.

1. Nematodes should first be killed in glacial acetic acid, AFA, or alcohol-glycerin.
2. Place nematodes in a small dish containing 70% alcohol–5% glycerin solution (several millimeters deep).
3. Partially cover the dish to allow gradual evaporation of the alcohol and water for approximately 24 to 36 h. The larger the nematodes, the longer the time needed to complete the evaporation.
   Note The evaporation procedure should be done slowly to prevent collapse of the worm; the larger the specimen, the longer the evaporation time.
4. Transfer the worms to another dish containing a few milliliters of glycerin. The specimen should be placed just below the surface; it will eventually (within a few hours) sink to the bottom of the dish. It is then ready to be mounted.
5. Place a drop of liquefied glycerin jelly on the slide, and transfer the specimen into the drop. When the coverglass is added, the glycerin jelly should flow out to the edges of the coverglass. Allow the glycerin jelly to begin to solidify, and apply the coverglass. If the specimen is large, place the coverglass onto small pieces of broken glass to provide more depth under the coverglass.
6. Allow the preparation to gel overnight (horizontal position). The following day, the coverglass can be sealed with Vaspar or enamel paint. These preparations usually last for several years, particularly if no unnecessary pressure is applied to the coverglass.

Note If personnel are not experienced in preparation of these permanent mounts, remember that the worms can be stored indefinitely in vials of pure glycerin. They can be studied in this medium as temporary mounts and then carefully returned to the vial of glycerin.

Glycerin

Nematode specimens can be examined as temporary mounts and subsequently stored in pure glycerin. This approach is particularly helpful for larger specimens (Fig. 9.8).

Lactophenol

Lactophenol is recommended when larger specimens are to be examined as temporary mounts. The worms can be placed into lactophenol from alcohol or formalin; clearing will occur, with the length of time depending on the size of the worm. The worms can then be washed in alcohol.

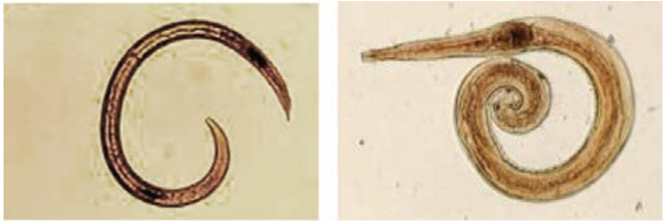
Lactophenol (Refractive Index, 1.44)

Glycerin, pure	20 ml
Lactic acid	10 ml
Phenol crystals, melted	10 ml
Distilled water	10 ml

Trematodes

Trematodes are usually studied as stained whole mounts (Fig. 9.9). Two stains that are recommended are carmine and hematoxylin. Many modifications are found in the literature, and most give excellent results. The stains are usually available commercially, and they can also be easily prepared in the laboratory.

Figure 9.8 Semipermanent mounts of small nematodes. (Left) Small nematode from soil sample. (Right) Male *Enterobius vermicularis* (pinworm) nematode.
doi:10.1128/9781555819002.ch9.f8



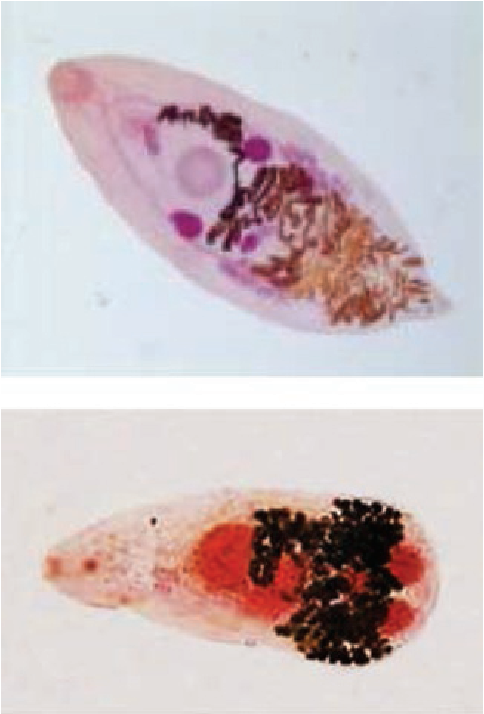


Figure 9.9 Stained trematodes. (Upper) *Eurytrema* sp. (Lower) *Heterophyes* sp. Note that the morphologic details are clearly visible after staining. doi:10.1128/9781555819002.ch9.f9

Semichon's Acid Carmine

Carmine powder	5 g
Distilled water	50 ml
Acetic acid, glacial	50 ml

Add glacial acetic acid slowly to the water. Add the carmine powder, and heat to 95 to 100°C for 15 min. Cool and filter (stock solution). Add few drops of stock solution to 70% alcohol, making the working stain pink.

Van Cleave's Combination Hematoxylin Stain

Van Cleave's combination hematoxylin stain is a combination of Delafield's hematoxylin and Ehrlich's hematoxylin that rarely overstains trematode specimens.

Delafield's Hematoxylin Stain

Hematoxylin powder	4 g
Ethyl alcohol, 95%	25 ml
Aluminum ammonium sulfate [$AlNH_4(SO_4)_2$], saturated aqueous solution (aluminum alum solution)	400 ml
Glycerin	100 ml
Methyl alcohol	100 ml

Dissolve the hematoxylin in ethyl alcohol, add aluminum alum solution, and let stand for 1 week exposed to air and light (in a paper-capped container). Filter; add glycerin and methyl alcohol. Age for 6 to 8 weeks in a tightly capped bottle in the refrigerator. This is the working stain solution (no dilution is needed).

Ehrlich's Hematoxylin Stain

Hematoxylin powder	2 g
Ethyl alcohol, 95%	100 ml
Glycerin	100 ml
Distilled water	100 ml
Acetic acid, glacial	10 ml
Potassium aluminum sulfate [$K_2SO_4Al_2(SO_4)_3{\cdot}12H_2O$] (potassium alum)	3 g

Dissolve hematoxylin powder in alcohol; add the other ingredients. Expose to air and light for at least 2 weeks (in a paper-capped container). The solution can be ripened immediately by the addition of 0.4 g of sodium iodate ($NaIO_3$). Store refrigerated in a tightly capped bottle. This is the working solution (no dilution is needed).

Van Cleave's Hematoxylin Staining Solution

Delafield's hematoxylin stain	1 ml
Ehrlich's hematoxylin stain	1 ml
Distilled water	100 ml
Potassium aluminum sulfate	6 g

Dissolve the potassium alum in water and add the two hematoxylin stains. This is the working solution.

1. Staining should be done in dilute solutions of carmine or hematoxylin (at least overnight). Specimens stained in carmine are placed into the dilute stain from 70% ethyl alcohol. Those stained in hematoxylin are placed in the stain from water. It is best to overstain and then destain (regressive stain approach).
2. Rinse in 70% ethyl alcohol.
3. Destain in weak acid-alcohol (2 to 4 drops of concentrated HCl in 100 ml of 70% ethyl alcohol). Leach the color from tissues until they are clear but the internal organs remain well stained. Destaining may take several minutes to hours; however, the specimens must be periodically checked to avoid overdestaining.
4. Rinse in 70% alcohol.
5. Place the specimens for 30 min to 1 h in a solution of 70% ethyl alcohol containing 1 or 2 drops of saturated aqueous Na_2CO_3, $NaHCO_3$, or $LiCO_3$. This step neutralizes the acid step and prevents continued destaining.
6. Rinse in 70% ethyl alcohol.

7. Dehydrate through 80, 95, and 100% ethyl alcohol, with 11 to 15 min for each alcohol change.
8. Clear the specimens in xylene for at least 15 min.
9. Mount in Permount or other permanent mounting medium.

Cestodes

Cestodes can also be examined as stained whole mounts, although the *Taenia* tapeworms can be examined more rapidly with India ink in a temporary mount (see chapter 4 for India ink injection of tapeworm proglottids). The same carmine and hematoxylin stains as used for trematodes can be used for cestodes.

India Ink Procedure for Tapeworm Proglottids

Identification of adult worms usually involves examination of a tapeworm proglottid. A *Taenia* proglottid must be gravid, containing the fully developed uterine branches. Using a syringe (1 ml or less) and a 25-gauge needle, India ink is injected into the central uterine stem of the proglottid, filling the uterine branches with ink, or into the uterine pore. The proglottid can then be rinsed in water or saline, blotted dry on paper towels, pressed between two slides, and examined. After the identification has been made, the proglottid can be left between the two slides (place a rubber band around the slides), dehydrated through several changes of ethyl alcohol (50, 70, 90, and 100%), cleared in two changes of xylene, and mounted with Permount for a permanent record. After the xylene step, the proglottid will be stuck to one of the two slides; do not try to remove it (it is very brittle and will crack), but merely add the Permount to the proglottid and then add the coverglass (Fig. 9.10).

Euparal Mounts of Tapeworm Proglottids

Most helminth eggs mounted in Euparal (Flatters and Garnett, Ltd., Manchester, UK) exhibit excellent optical and drying properties, and this mounting medium can also be used to mount tapeworm proglottids (25). Proglottids should be placed in 100% ethyl alcohol for 5 to 10 min and then pressed between two slides as described above. They should then be placed in Euparal; transparency will occur in 2 to 3 h. The best results have been obtained by keeping the slides in a 50°C incubator overnight.

Double-Coverglass Method for Microscopic Mounts of Cysts, Eggs, and Larvae

Slides prepared by the double-coverglass technique will last for several weeks to several years, depending on the organisms and the care taken in preparation. The procedure is as follows (16).

1. Place a small drop of 10% formalin or formalin-saline suspension (containing cysts, eggs, and/or larvae) in the center of a 22-mm round or square coverglass.

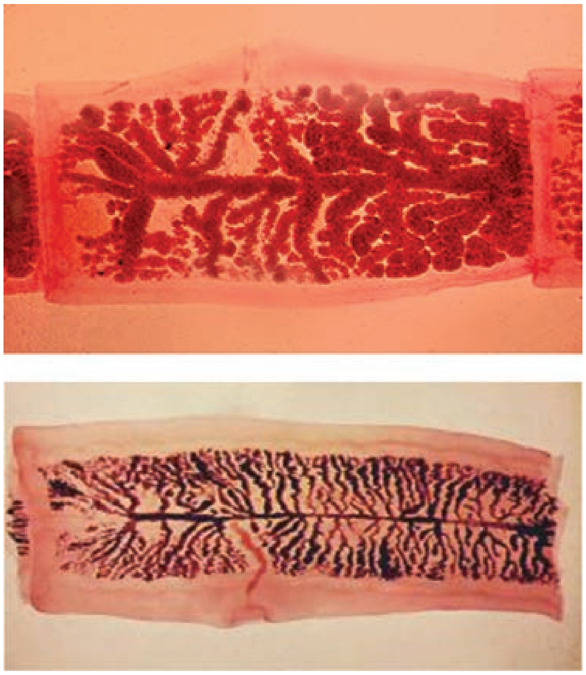


Figure 9.10 *Taenia* gravid proglottids. (Upper) *Taenia solium* stained with carmine stain. (Lower) *Taenia saginata* after India ink injection of the uterine branches.
doi:10.1128/9781555819002.ch9.f10

2. Using forceps, very carefully apply a smaller coverglass (12, 15, or 18 mm round or square) to the suspension so that the fluid flows to the edges of the small coverglass (with no bubbles). If excess fluid flows beyond the small coverglass, it should be blotted dry or allowed to evaporate. Do not allow it to dry too long; otherwise, bubbles will accumulate under the coverglass.
3. Place a large drop of Permount or other permanent mounting medium in the center of a 1- by 3-in. (1 in. = 2.54 cm) microscope slide. Using forceps, place the double-coverglass preparation (small coverglass side down) onto the mounting medium so that the medium flows to the edges of the large coverglass.
4. Allow the preparation to thoroughly dry in a horizontal position (this may take several days).

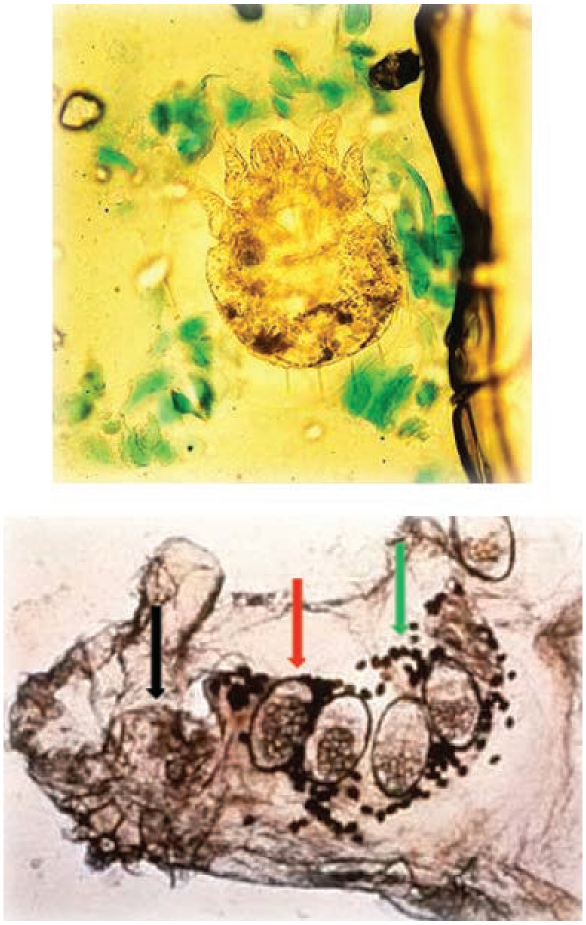
Mounting of Arthropods for Examination (20, 26–28)

Before being mounted, specimens in which xylene is used as a solvent require dehydration through 50, 70, and 95% ethyl alcohol. Clearing can be done in clove oil, carbol-xylene (3 parts xylene to 1 part phenol crystals), or absolute ethyl alcohol followed by xylene. The specimens should remain in each solution for at least 15 to 20 min. Specimens mounted in balsam or Permount will take several days to harden; however, those mounted in isobutyl methacrylate will dry very rapidly (within a few hours). With this type of permanent mount, ringing or sealing of the coverglass is not necessary. Some specific recommendations are presented below.

Mites

Temporary mounts can be made with a drop of 50% ethyl alcohol, which is gently heated. Clearing and extension of the specimen occur, revealing the typical morphology (Fig. 9.11). Permanent mounts of living specimens can be made by placing the specimen in a drop of chloral-gum medium, adding a coverglass, and then gently heating until bubbling begins. Specimens originally preserved in alcohol must first be washed in distilled water to remove the alcohol.

Figure 9.11 *Sarcoptes scabiei* itch mites in wet mounts. (Upper) Adult mite. (Lower) Skin scraping containing adult mite (black arrow), eggs (red arrow), and mite feces (green arrow). These specimens could be seen using the high dry objective (magnification, ×400). (Lower image courtesy of National Institutes of Health, http://www.nlm.nih.gov/medlineplus/ency/images/ency/fullsize/2471.jpg). doi:10.1128/9781555819002.ch9.f11



Chloral-Gum Medium

Distilled water	35 ml
Chloral hydrate	30 g
Gum arabic	20 g
Glycerin	12 ml
Glucose syrup (e.g., Karo syrup)	3 ml

Fleas and Lice

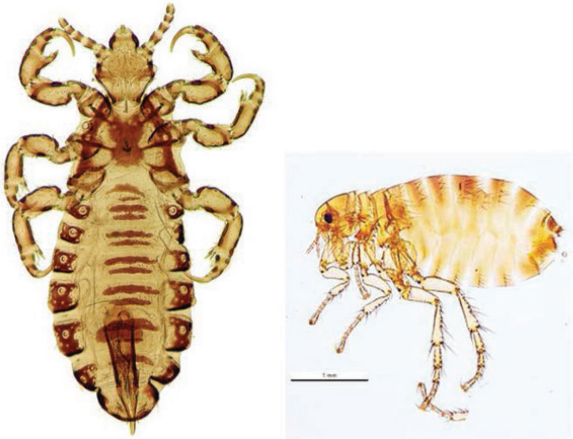
Specimens may be preserved in 70% alcohol or mounted on slides for identification (Fig. 9.12).

1. Drop living fleas or preserved specimens into 10% KOH for a few days until sufficiently cleared.
2. Transfer for 30 min to a small volume of water containing a few drops of concentrated HCl.
3. Dehydrate in 50% ethyl alcohol for 30 min.
4. Dehydrate in 95% ethyl alcohol for 30 min.
5. Clear in beechwood creosote for 1 h, or place in several changes of absolute ethyl alcohol and clear in clove oil or xylene.
6. Mount on slides in balsam, Permount, or isobutyl methacrylate.

Ticks

Specimens should be preserved in 70% alcohol or cleared and mounted on slides. They can be fixed in an extended position by being gently pressed between two slides while immersed in hot water. Clearing in KOH, dehydration in

Figure 9.12 (Left) Body louse, *Pediculus humanus*. (Right) Flea, *Pulex irritans*. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch9.f12



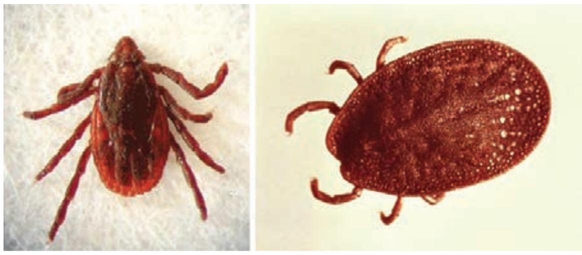


Figure 9.13 Ticks. (Left) Example of a hard tick, *Ixodes* sp. (Right) Example of a soft tick, *Argas* sp. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch9.f13

alcohols, and mounting with balsam, Permount, or isobutyl methacrylate can be done as recommended for fleas (Fig. 9.13).

Miscellaneous Arthropods

Spiders, scorpions, centipedes, lice, bedbugs, maggots and other larvae, nymphs, and soft-bodied insects can be preserved in 70% ethyl alcohol containing a small amount of glycerin to prevent drying and shrinkage. Containers should remain tightly sealed and should be checked periodically. Larger, hard-bodied insects can be pinned, labeled, and stored in boxes containing naphthalene flakes or paradichlorobenzene to prevent damage from mold and living insects (Fig. 9.14) (29).

Figure 9.14 Example of large hard-body insects, which are triatomid bugs. These large insects can be pinned, labeled, and stored in boxes containing naphthalene flakes or paradichlorobenzene. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch9.f14

Note Identification of the many species of arthropods can best be handled by an entomologist or specialist working with a particular arthropod group. Additional help with identification may be obtained at a local university entomology department, a military base (over 100 entomologists are employed by the U.S. Army and Navy), or the entomology department of natural history museums or the Smithsonian Institution (30).

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10

Artifacts That Can Be Confused with Parasitic Organisms

Protozoa
Amebae
Flagellates
Ciliates
Coccidia and microsporidia
Cryptosporidium spp. and *Cyclospora cayetanensis*
Cystoisospora belli
Microsporidia
Blood and body fluids
Malaria parasites and *Babesia* spp.
Leishmaniae and trypanosomes
Microfilariae
Body fluids: ciliated epithelial cells
Helminths
Adult worms and larvae
Eggs
Human cells
Polymorphonuclear leukocytes
Eosinophils
Macrophages
Lymphocytes
Red blood cells
Charcot-Leyden crystals
Nonhuman elements seen in feces (yeast cells)
Insect larvae
Spurious infections
Delusory parasitosis (delusionary infestation)

Although many body sites and specimens can be examined for the presence of parasites, the most difficult specimen in which to differentiate parasites from artifacts is usually fecal material. Feces consist of a number of components, including (i) undigested food residue; (ii) digestive by-products; (iii) epithelial and other human cells, mucus, and other secretions from the digestive tract; and (iv) many types of microorganisms such as bacteria and yeasts. Considering the ratio between fecal debris and parasites, it is not surprising that many artifacts are responsible for incorrect identifications of protozoan trophozoites and cysts and of helminth eggs and larvae. Often, many yeast cells and other artifacts are confused with coccidian oocysts or microsporidial spores. Appropriate training, adherence to protocols, use of quality control measures, and availability of reference materials and consultants should help minimize identification errors.

Protozoa

A number of cells and other organisms can easily be confused with intestinal protozoa. These are listed in Tables 10.1 and 10.2 and illustrated in Fig. 10.1.

Amebae

Occasionally, free-living amebae are found in feces or as contaminants in water. Morphologically, they differ from parasitic amebae in having one or more large contractile vacuoles in the trophozoite form and having very thick cyst walls. They can also be differentiated on the basis of cultivation; i.e., they are much easier to culture than the pathogenic protozoa. Amebae which have been recovered from stool material include *Entamoeba moshkovskii*, *Naegleria gruberi*, *Hartmanella hyalina*, *Sappinia diploidea*, *Vahlkampfia punctata*, and *Vahlkampfia lobospinosa*. Contamination of specimens can be avoided by using dry collection containers, saline, or formalin for the concentration rinses and dilution of the specimen and by rapid fixation or examination of the specimen immediately after passage (1).

Some protozoa, such as *Entamoeba coli*, may contain fungi. *Sphaerita* spp. can be found in the cytoplasm, and *Nucleophaga* spp. can be found in the nucleus. *Sphaerita* spp. (sometimes called *Polyphaga* spp.) measure approximately 0.5 to 1.0 µm and are found in tightly packed clusters (Fig. 10.2) (2).

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TABLE 10.1 Artifacts and other confusing cells and organisms[a]

Artifact	Resemblance	Differential characteristics of artifacts (permanent stain)		
		Saline mount	Cytoplasm	Nucleus
PMNs[b] (seen in dysentery and other inflammatory bowel diseases)	*E. histolytica*/*E. dispar* cysts	Usually not a problem if cells are from fresh blood. Granules in cytoplasm. Cell border irregular.	Less dense, often frothy. Border less clearly demarcated than that of ameba. May look very similar to that in protozoa.	More coarse. Larger, relative to size of organism. Irregular shape and size. Chromatin unevenly distributed. Chromatin strands may link nuclei or may appear to be four separate nuclei as the cell ages.
Macrophages (seen in dysentery and other inflammatory bowel diseases; may be present in purged specimens)	Amebic trophozoites, especially *E. histolytica*/*E. dispar*	Nuclei larger and of irregular shape, with irregular chromatin distribution. Cytoplasm granular; may contain ingested debris. Cell border irregular and indistinct. Movement irregular; pseudopodia indistinct but may mimic protozoa.	Coarse. May contain inclusions. May include RBCs; if RBCs present, will mimic *E. histolytica*.	Large and often irregular in shape. Chromatin irregularly distributed. May appear to have karyosome. Will have more nuclear material per cell than protozoan trophozoite (nucleus may be absent).
Squamous epithelial cells (from anal mucosa)	Amebic trophozoites	Nucleus refractile and large. Cytoplasm smooth. Cell border distinct.	Stains poorly	Large and single. Large chromatin mass may resemble karyosome.
Columnar epithelial cells (from intestinal mucosa)	Amebic trophozoites	Nucleus refractile and large. Cytoplasm smooth. Cell border distinct.	Stains poorly	Large with heavy chromatin on nuclear membrane. Often large central chromatin mass resembling karyosome.
Blastocystis spp. (protozoan) Central body form (most common form seen in stool)	Protozoan cysts of other species or yeasts	Spherical to oval, 0.6–15 µm long. Central clear area. Peripheral nuclei may be confusing.	Central mass may stain light or dark. Prominent wall.	Peripheral nuclei may look like large karyosomes (no peripheral nuclear chromatin).
Yeasts (normal constituent of feces)	Protozoan cysts, microsporidia	Oval. Thick wall. No internal structure. Budding forms may be seen. Some are round and overlap size of protozoan cysts. Very small forms will mimic microsporidial spores.	Oval to round. Little internal structure. Refractile cell wall. Budding forms may be seen; single small yeast cells will resemble microsporidia.	None
Bacteria (normal constituent of feces)	Microsporidia	Small, oval; no internal structure. May have terminal spores that resemble vacuoles in microsporidian spore.	Various colors on modified trichrome stains; generally pink and will resemble large, Gram-negative rods that mimic microsporidian spores.	None
Starch granules	Protozoan cysts	Rounded or angular. Very refractile. No internal structure. Stain pink to purple in iodine mounts.	Not a problem in permanently stained slides.	None
Pollen	Helminth eggs	Often very precise morphology; little to no internal structure. Often stain very dark in trichrome stains.	Morphology can usually be differentiated from true helminth eggs; comparative images are very helpful.	None

[a] Other artifacts, such as contaminating plant cells, are occasionally seen. These should not be difficult to differentiate.

[b] Stools containing many human cells are usually soft or diarrheic; consequently, they would be unlikely to contain amebic cysts, and cells containing what appear to be four nuclei are almost certain to be PMNs.

TABLE 10.2 Artifacts that resemble parasites

Clinical specimen and artifact	Resemblance
Stool	
Free-living amebae, flagellates, ciliates	Parasitic amebae, flagellates, ciliates
Free-living helminths, helminth eggs, or mite eggs	Helminth eggs, larvae, or adult worms
Yeast cells	*Cryptosporidium* spp., *Cyclospora* spp., microsporidia, helminth eggs, or protozoan cysts (depending on size and stained colors)
Bacteria	Microsporidian spores
Fungi	Helminth eggs
Plant material	
Cells	Protozoan cysts, helminth eggs
Root hairs	Nematode larvae
Pollen grains	Helminth eggs (*Ascaris* or *Taenia* spp.)
Pineapple juice crystals	Charcot-Leyden crystals
Human cells	
PMNs	
Fresh bleeding (fresh cells)	Regular PMNs as in peripheral blood smear
Old blood (disintegrating cells)	*Entamoeba histolytica*/*E. dispar*[a] cyst
Macrophages	*Entamoeba histolytica*/*E. dispar*[a] trophozoite
RBCs	*Cryptosporidium* spp., *Cyclospora* spp.
Epithelial cells	Amebic trophozoites
Blood	
Platelets	Malaria parasites, *Babesia* spp.
Abnormal RBC inclusions (Howell-Jolly bodies, Cabot's rings)	Malaria parasites, *Babesia* spp.
Contaminants	
Yeast	Fungemia, parasitemia
Bacteria	Bacteremia
Plant or dust fibers	Microfilariae
Stain precipitate	Malaria, *Babesia* spp.
Body fluids	
Detached ciliary tufts, ciliocytophthoria	Flagellate or ciliate protozoa
Respiratory specimens	
Yeast	*Pneumocystis jirovecii*, *Cryptosporidium* spp.
Detached ciliary tufts, ciliocytophthoria	Flagellate or ciliate protozoa
Urine	
Pentatrichomonas hominis (from stool contamination)	*Trichomonas vaginalis*
Bacteria	Microsporidian spores

[a] *Entamoeba moshkovskii* and *E. bangladeshi* also resemble *Entamoeba histolytica* and *E. dispar* but are much less common.

Flagellates

Flagellates can be difficult to differentiate, and free-living organisms are occasionally seen in a stool specimen that has been contaminated with water or saline containing *Bodo caudatus* and *Cercomonas longicauda*. These two organisms are classified in the same family as *Retortamonas intestinalis* (Fig. 10.3).

Ciliates

Apparently free-living ciliates are found in stagnant water, sewage, and soil and may be seen in fecal specimens contaminated with water or saline. Organisms that have been reported are *Litonotus* and *Paramecium* (1–3) (Fig. 10.4).

Coccidia and Microsporidia

Cryptosporidium spp. and *Cyclospora cayetanensis*

Cryptosporidium measures approximately 4 to 6 μm and overlaps in size with a number of objects, including yeasts and debris that are found in stool specimens. *Cyclospora cayetanensis* measures approximately 8 to 10 μm and can be easily confused with other coccidia or artifacts, especially if careful measurements are not taken. Without the use of modified acid-fast stains or immunoassay detection methods, a light infection with coccidia will probably be missed; the more normal the stool consistency, the fewer oocysts and more artifacts will be present. The oocysts can also be confused with artifact material when the staining results are of poor quality; they appear nonuniform and more like debris (Fig. 10.1).

Cystoisospora belli

When *Giardia lamblia* shrinks within the cyst wall, it can resemble *Cystoisospora belli* in the immature form (a single developing sporoblast; the two mature sporocysts within

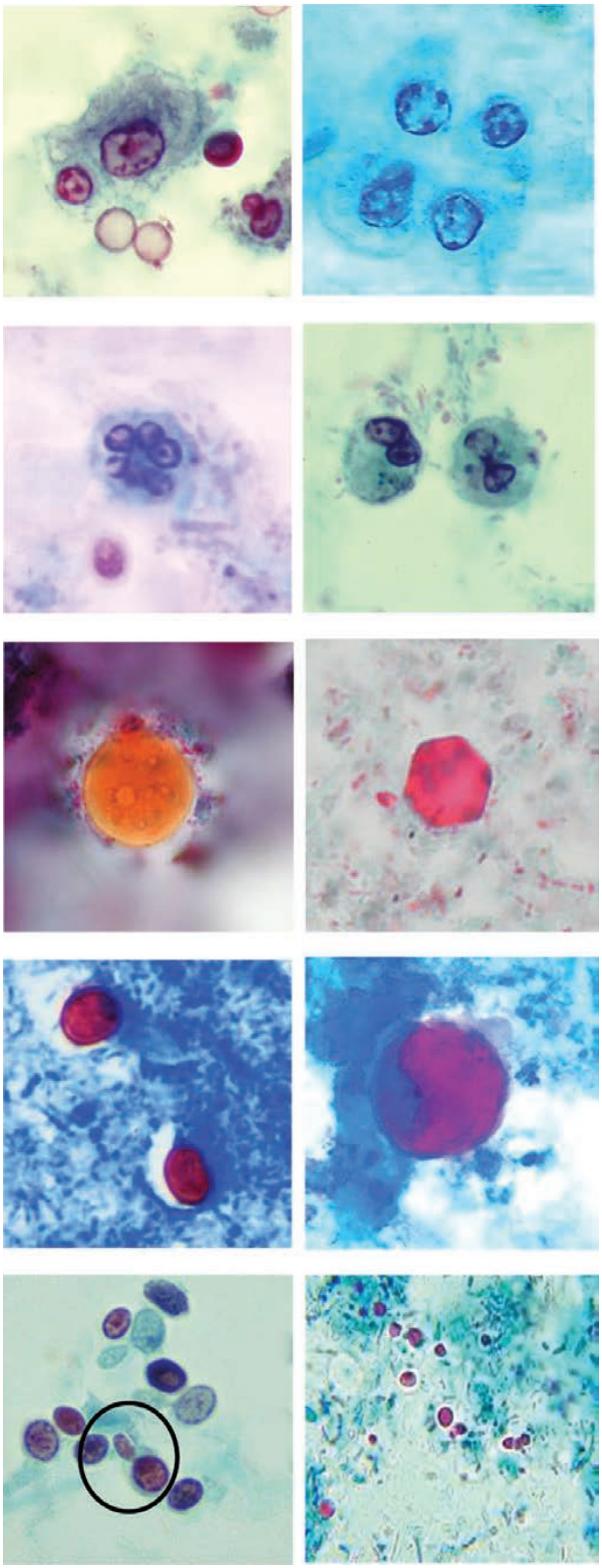


Figure 10.1 Various structures that may be seen in stool preparations. (Top row) Macrophage (left) and epithelial cells (right) that can be confused with *Entamoeba histolytica/E. dispar* trophozoites. (Second row) PMN with a fragmented nucleus (left) and artifact (right) that can be confused with *Entamoeba* spp. cysts. (Third row) Two artifacts that can resemble protozoan cysts. (Fourth row) Yeast cells (left) and an artifact (right) that can be confused with *Cryptosporidium* spp. and *Cyclospora cayetanensis*, respectively, on positive acid-fast stains; it is important to measure the structures and organisms carefully before confirming organism identification. (Bottom row) Yeast cells (left) that can be confused with microsporidial spores (however, notice the budding cell within the circle), and artifacts (right) that can also be confused with microsporidial spores; these were thought to be small yeast cells. doi:10.1128/9781555819002.ch10.f1

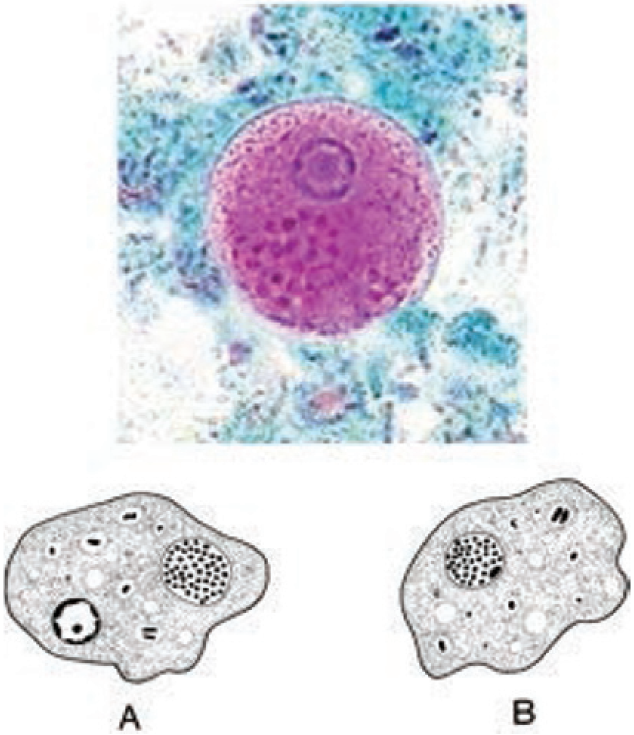


Figure 10.2 Fungi parasitizing *Entamoeba coli*. (Upper) *Entamoeba coli* containing ingested *Sphaerita*. (Lower) (A) *Sphaerita* (or *Polyphaga*) sp. within the cytoplasm; (B) *Nucleophaga* sp. within the nucleus. doi:10.1128/9781555819002.ch10.f2

the oocyst wall have not yet formed). Although this error is occasionally made, the easiest way to differentiate the two is by size. *Giardia* cysts measure approximately 11 to 14 µm long by 7 to 10 µm wide, and *C. belli* measures 20 to 33 µm long by 10 to 19 µm wide. This represents another situation in which measurement of the organisms can help prevent diagnostic errors (Fig. 10.5).

Microsporidia

Microsporidian spores in humans measure approximately 1 to 4 µm, with the majority being 1 to 2 µm. These spores are round to oval and can mimic yeast cells and bacteria. Although they do stain in a modified trichrome procedure (see chapter 3), the color is usually pale and both the size and color overlap with those of many of the yeasts or bacteria present in the specimen (Fig. 10.1). The spores usually stain light to dark pink; they can be confused with bacteria, looking like large Gram-negative rods. Also, some of the bacilli contain terminal spores that mimic the large vacuole often seen at one end of the microsporidian spore. Size and staining color are rarely that helpful. It is important to prepare very thin smears prior to staining; the spores

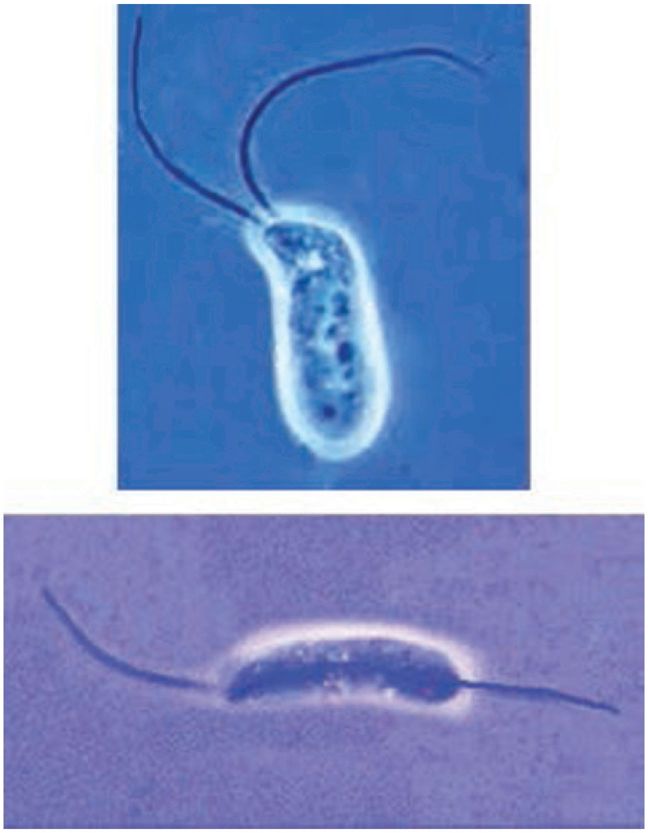
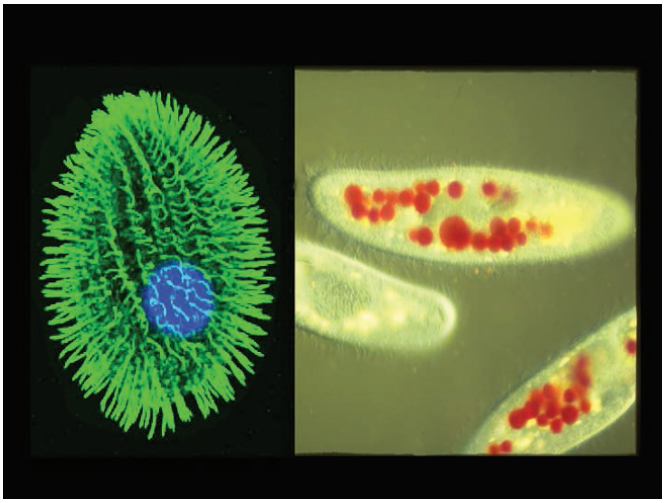


Figure 10.3 Free-living freshwater flagellates. (Upper) *Bodo caudatus*. (Lower) *Cercomonas longicauda*. These organisms can be confused with parasitic protozoa, particularly if motility is seen in a wet mount of fresh stool contaminated with freshwater. doi:10.1128/9781555819002.ch10.f3

Figure 10.4 Free-living freshwater ciliates. (Left) *Tetrahymena*. (Right) *Paramecium*. (Courtesy of David Nelson.) doi:10.1128/9781555819002.ch10.f4



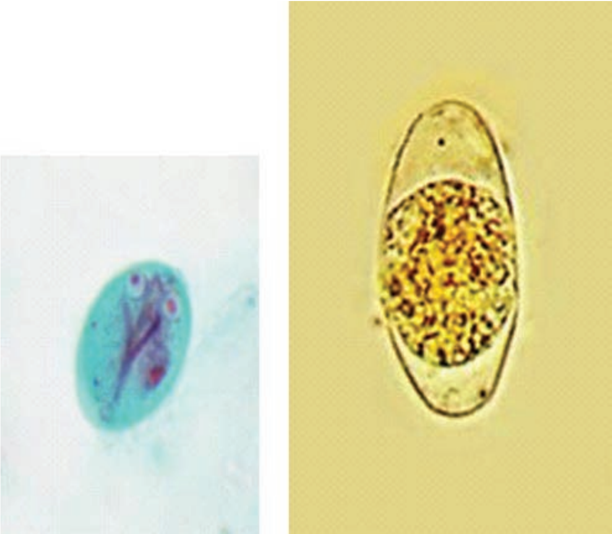


Figure 10.5 *Giardia lamblia* and *Cystoisospora belli*. (Left) *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*), trichrome stain. (Right) *Cystoisospora belli*, iodine wet mount. Note the size differences of the two organisms. doi:10.1128/9781555819002.ch10.f5

will be more visible, and the stain will penetrate more successfully. Without confirmation of the presence of the polar tubule in at least some of the spores (diagonal or horizontal line across the spore), it is almost impossible to confirm a microsporidial infection by using routine modified trichrome stains for fecal material. The microsporidia require molecular testing or electron microscopy to confirm the genus/species designations.

Blood and Body Fluids

Malaria Parasites and *Babesia* spp.

One of the most common errors in examining blood smears is the incorrect identification of platelets as parasites. When mature schizonts rupture, the merozoites almost immediately penetrate another red blood cell (RBC) and are not seen outside of the RBCs. Extracellular "organisms" are almost always platelets. It is important to focus constantly when examining blood smears; the platelets are often on top of the RBCs. Another tip relates to color; parasites always have two colors, blue nuclei and red cytoplasm, and they are separate. Platelets tend to be uniform in color with no real internal structure; there are red and blue components, but the colors almost blend to form purple. Other internal structures within the RBCs such as Howell-Jolly bodies and Cabot's rings may be confusing (Fig. 10.6 to 10.8). If the blood is held too long in EDTA anticoagulant or the ratio of blood to anticoagulant is incorrect, *Plasmodium* organisms can become distorted and may resemble other malaria stages or different species. Potential problems with using EDTA anticoagulant for the preparation of thin and thick blood films are discussed in chapter 31 (see Table 31.2).

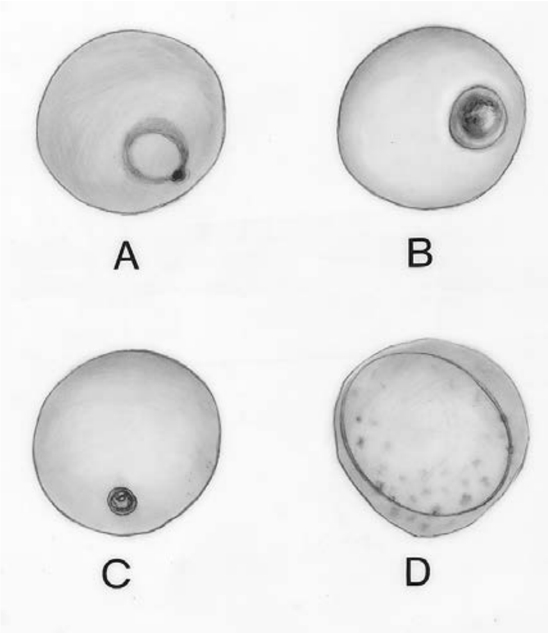


Figure 10.6 Various structures within the RBC. (A) Malarial "ring" form (early trophozoite); (B) platelet on the RBC surface; (C) Howell-Jolly body; (D) Cabot's ring. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch10.f6

If a tube of blood containing EDTA cools to room temperature and the cap has been removed, the parasites can undergo several changes. The parasites within the RBCs will respond as if they were now in the mosquito after being taken in with a blood meal. The morphology of these changes in the life cycle and within the RBCs can cause confusion when examining blood films prepared from this blood (Fig. 10.8).

Leishmaniae and Trypanosomes

Although they are rarely seen, intracellular amastigotes called Leishman-Donovan bodies may be found in the monocytes in peripheral blood smears from patients with visceral leishmaniasis. When they are found in a bone marrow or splenic aspirate preparation, they are easier to find, probably because at that point there may be a high index of suspicion regarding the etiologic agent. In any suspected case of visceral leishmaniasis, peripheral blood buffy coat preparations are usually examined before more invasive procedures are undertaken. The amastigotes range from 3 to 5 µm, and a defined nucleus and kinetoplast may be difficult to see, particularly if a number of organisms are packed in the cell (see chapters 7 and 19). The amastigotes

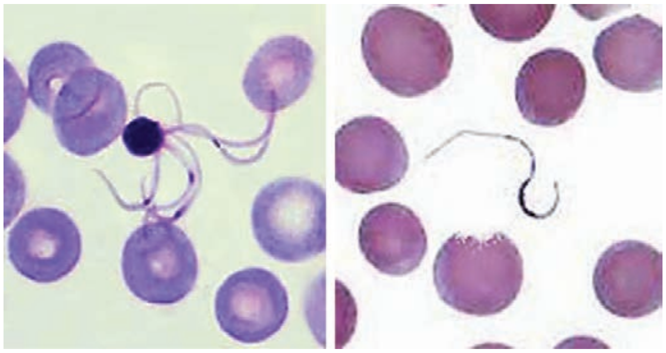


Figure 10.8 Exflagellation of *Plasmodium vivax* microgametocyte; these microgametes could easily be confused with some type of spirochete. These forms were seen in blood films prepared from blood stored for longer than 12 h in EDTA prior to additional smear preparation. (Left) Male *Plasmodium* gametocyte (microgametocyte) undergoing exflagellation. (Right) Single strand—very easily resembles a spirochete.
doi:10.1128/9781555819002.ch10.f8

may resemble *Histoplasma capsulatum* if the kinetoplast bar structure is not easily seen (Fig. 10.9).

Microfilariae

Any laboratory using staining reagents must use good quality control measures to ensure that the solutions do not become contaminated with artifacts or free-living organisms. Bits of cotton fiber, lint, and other components of dust can mimic microfilariae in wet mounts or when stained. However, the artifacts do not contain any internal nuclei. Rarely, nonhuman parasites can also be confused if they appear on the stained blood smears. One laboratory used to dry the blood films upright, leaning against a fish tank (many years ago, before more stringent safety measures were instituted). Examination of one of the stained blood films suggested that the patient had a filarial infection. Since the patient history did not support this diagnosis, further studies were performed. When samples of the fish tank water were centrifuged and examined,

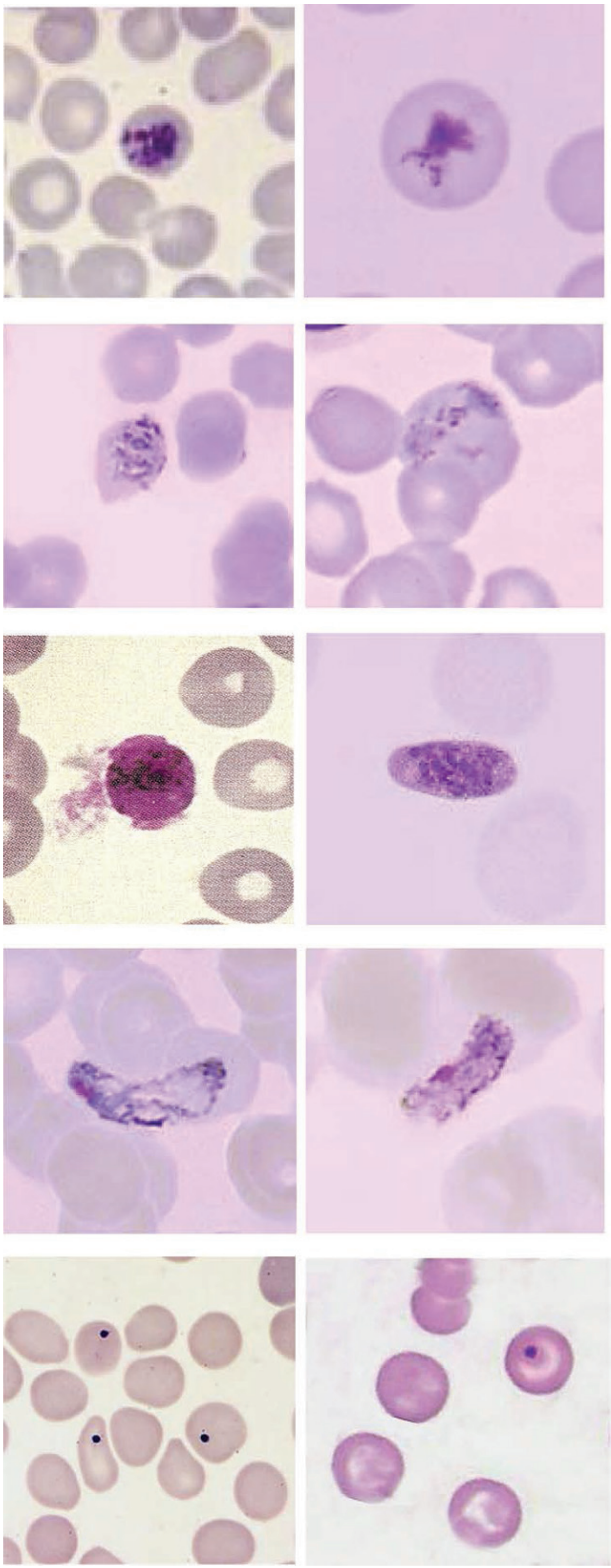


Figure 10.7 (Top row and second row) Stain deposition on the surface of uninfected RBCs that could easily be confused with developing *Plasmodium* spp. stages. (Third row) *Plasmodium falciparum* gametocytes that have rounded up and no longer appear as the typical crescent-shaped gametocytes that are normally seen (could be due to low temperatures and/or storage for too many hours in EDTA blood). (Fourth row) Developing *Plasmodium vivax* trophozoites that appear to resemble *P. falciparum* gametocytes (found on blood smears prepared from EDTA blood that had been collected more than 8 h previously). (Bottom row) RBCs containing Howell-Jolly bodies that could be confused with very small, young ring forms of *Plasmodium* spp.
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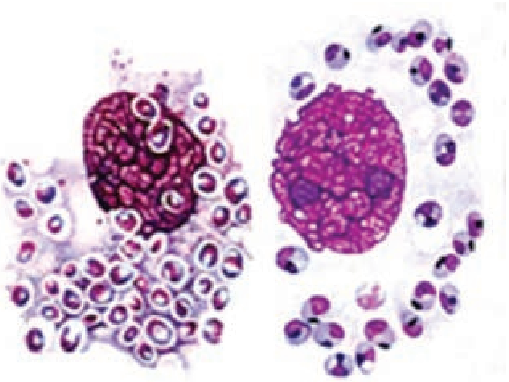


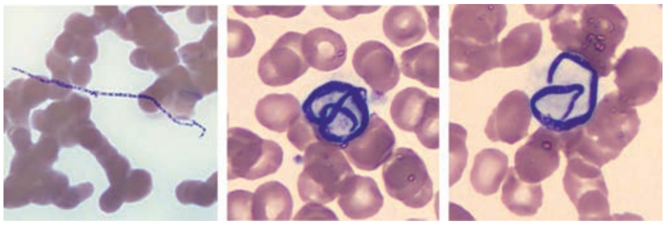
Figure 10.9 (Left) *Histoplasma capsulatum*. (Right) *Leishmania donovani*. Note that the *Leishmania* amastigotes have the bar structure while the *Histoplasma* amastigotes do not; *Histoplasma* also has a "halo" around the organisms. doi:10.1128/9781555819002.ch10.f9

the "microfilariae" were found! This is just one example of the many unusual sources of artifact contamination (Fig. 10.10).

Body Fluids: Ciliated Epithelial Cells

Detached ciliary tufts (ciliocytophthoria) have been seen in a variety of body fluids (especially peritoneal and amniotic fluids; also respiratory specimens). These tufts are the remnants of ciliated epithelium that are found as a part of normal cellular turnover in a number of sites: respiratory tract and sinuses, ventricles of the brain, central canal of the spinal cord, and epithelia of the male and female reproductive tracts. The tufts are motile, measure 10 to 15 µm in diameter, and can be confused with ciliated or flagellated protozoa. However, when they are carefully examined on a stained smear, there is no internal structure like that seen in protozoa (4–6). Ciliated epithelium cells can be seen in Fig. 10.11; ciliocytophthoria are anucleate remnants of these ciliated cells. However, in some cases, the cell nuclei are clearly visible. The ciliated tufts can be seen on the cells in this figure.

Figure 10.10 Fungal spores (*Helicosporium* type). Artifacts that can resemble microfilariae in stained blood films; these structures are not parasites but instead are some type of thread. Note that there is little to no internal structure visible. (Left) Courtesy of the CDC Public Health Image Library. (Middle and right) Photographed at a higher magnification than the image on the left. doi:10.1128/9781555819002.ch10.f10



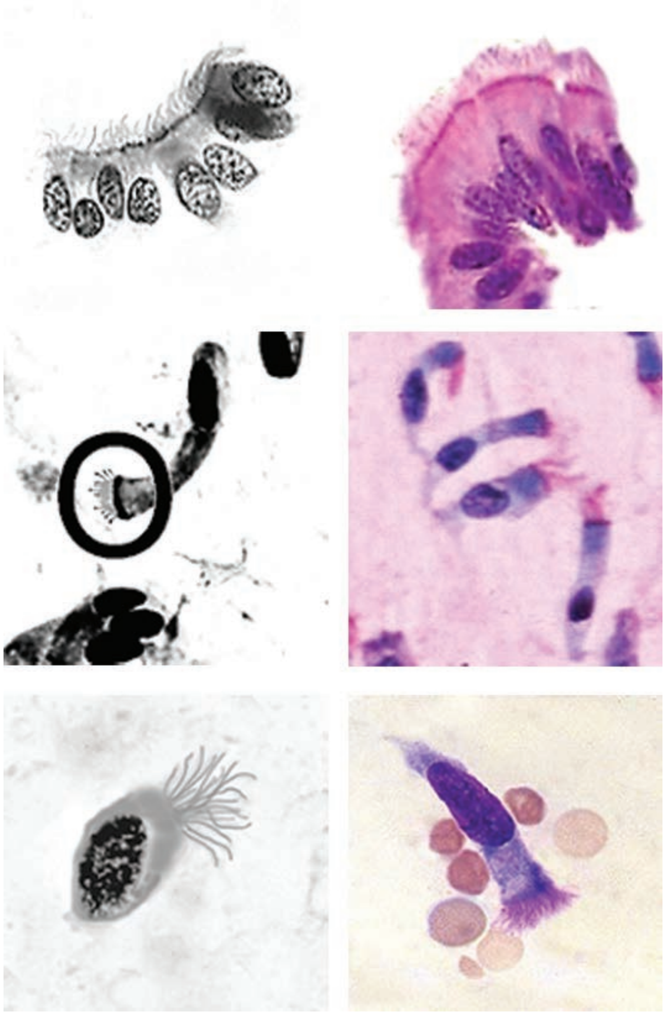


Figure 10.11 Bronchial epithelium cells. When these cells disintegrate, the ciliary tufts may be visible and may be confused with protozoan flagellates or ciliates (detached ciliary tufts = ciliocytophthoria). doi:10.1128/9781555819002.ch10.f11

These cells have been found in many clinical specimens, including peritoneal fluid (7). Recognition of these uncommon structures can be important in avoiding a misdiagnosis with a suspect protozoan infection. This has also been recognized as a potential problem in the virology laboratory (5). The difference between ciliocytophthoria and ciliated or flagellated parasites becomes very important during examination of specimens submitted for cytologic testing. Also, in performing immunofluorescence assays (direct fluorescent antibody [DFA] tests) used in the virology laboratory for the rapid detection of viruses, assessment of the cellularity of specimens is required for the most effective use of the DFA assay.

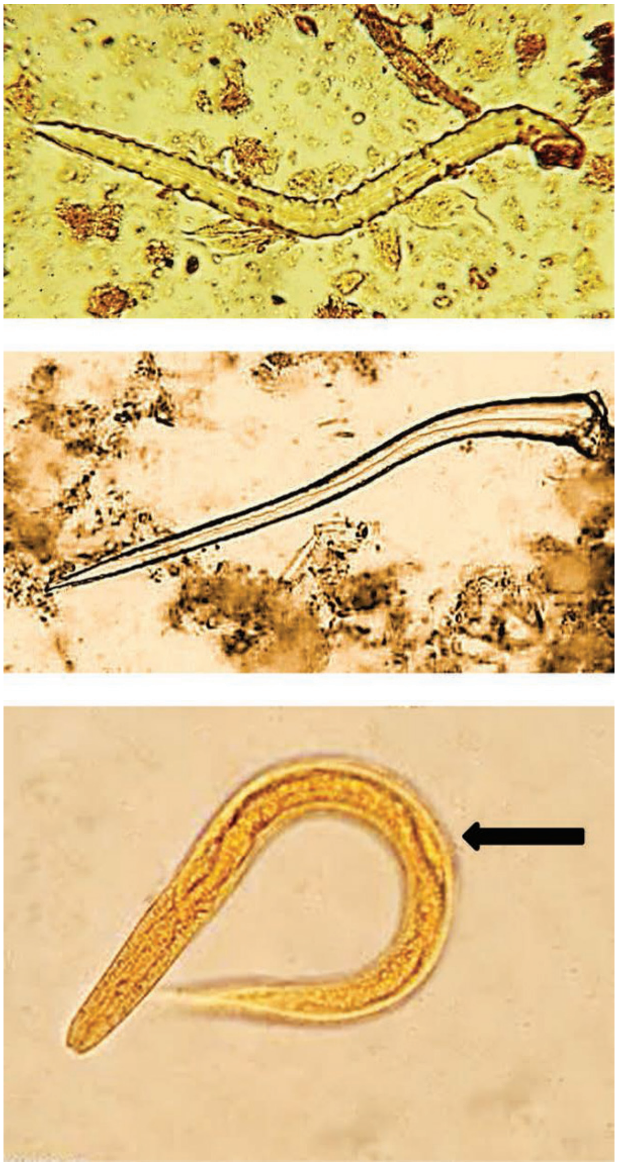


Figure 10.12 (Top) Root hair. (Middle) Root hair. Note that there is no internal structure visible within the root hairs. (Bottom) *Strongyloides stercoralis* rhabditiform larva. Note the short buccal cavity at the head end of the larva and the genital primordial packet of cells within the curved portion of the body (arrow). doi:10.1128/9781555819002.ch10.f12

Helminths

Adult Worms and Larvae

Plant or root hairs, such as the fuzz on peaches, may resemble nematode larvae. The root hairs tend to be clear and refractile, while the larvae pick up stain (iodine) which reveals internal structures (Fig. 10.12). It is important to recognize this potential error when examining formalin-fixed specimens submitted as proficiency testing specimens. In fecal specimens from patients with diarrhea, partially digested plant material, such as bean sprouts or other vegetable material, can mimic adult nematodes or tapeworm proglottids. Also, all stages of free-living nematodes can occur in feces or as contaminants of the water used in making fecal suspensions.

Hairworms (often called horsehair worms) belonging to the phylum Nematomorpha can be confused with human parasites (8). These adult worms are slender, measuring 10 to 50 cm long, and have a blunt, rounded anterior end. “The caudal end of the male is bifurcate or has a dorsoventral groove; that of the female is entire or trilobate” (8) (Fig. 10.13). The adult worms are free living in water, while the larvae are parasites of insects. Human infection is quite rare and accidental, although in the past literature, serious health problems were attributed to “hair snakes” in the human body. Generally, human infection occurs through ingestion of free-living adult worms or adolescent worms within their insect hosts in drinking water or food. Worms have been reported as passing from the urethra in several cases. They have been identified as being in the genera *Gor-*

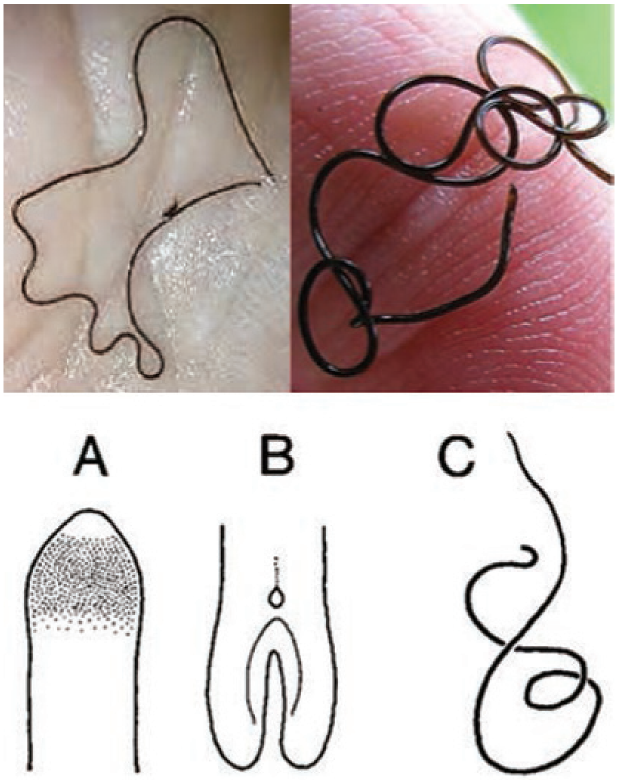


Figure 10.13 *Gordius* worms. (Upper) Adult worms (called hairworms or horsehair worms), which measure 20 to 50 cm long and are very slender. (Lower) Characteristic structure of *Gordius* worms. (A) Diagram of anterior end; (B) diagram of posterior end of a male worm in the genus *Gordius*; (C) whole worm. Bar, 1 cm. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch10.f13

dius, *Chordodes*, *Parachordodes*, *Paragordius*, *Pseudogordius*, and *Neochordodes*. Worms have been recovered in vomitus, urine, and feces; often, the stated origin of the worms in the body was not well documented. In spite of the reported cases, no evidence of pathogenicity has been demonstrated (8). Often symptoms were attributed to other causes or were psychological.

In some cases, very large objects are recovered in patient stools. One structure resembled a trematode, but was actually a slug. These objects can be very confusing, particularly when it is unclear whether the object actually passed through the intestinal tract or entered the stool after passage (Fig. 10.14).

Eggs

Plant cells tend to have thick, smooth walls and are not as symmetrical as helminth eggs (Fig. 10.15 and 10.16). Some of these plant cells range from 15 to 150 μm in diameter and may be confused with *Ascaris* eggs. Pollen grains are also thick-walled, symmetrical structures that stain very darkly with iodine, may be round or trilobed, and are 15 to 20 μm in diameter (Fig. 10.17). They may resemble *Taenia* eggs. Another example is the "Beaver body." This structure is *Psorospermium haeckelii*, a stage of an alga that occurs in the tissues of crayfish. It is sometimes confused with helminth eggs when found in fecal specimens from individuals who have ingested crayfish (9) (Fig. 10.18). Accidental ingestion or contamination of food or water containing the eggs of plant nematodes, such as *Heterodera* species or mites or mite eggs, can also lead to confusing situations.

Human Cells

The human cells most likely to cause problems with identification are the polymorphonuclear leukocytes (PMNs) and the macrophages seen in stained fecal smears (Table 10.1; Fig. 10.19). These cells are frequently present in patients with nonspecific inflammatory bowel disease (ulcerative colitis). Therapy for this condition often includes immunosuppressive agents; this therapy would definitely be contraindicated in patients with amebiasis (2). Therefore, the correct identification between human cells and

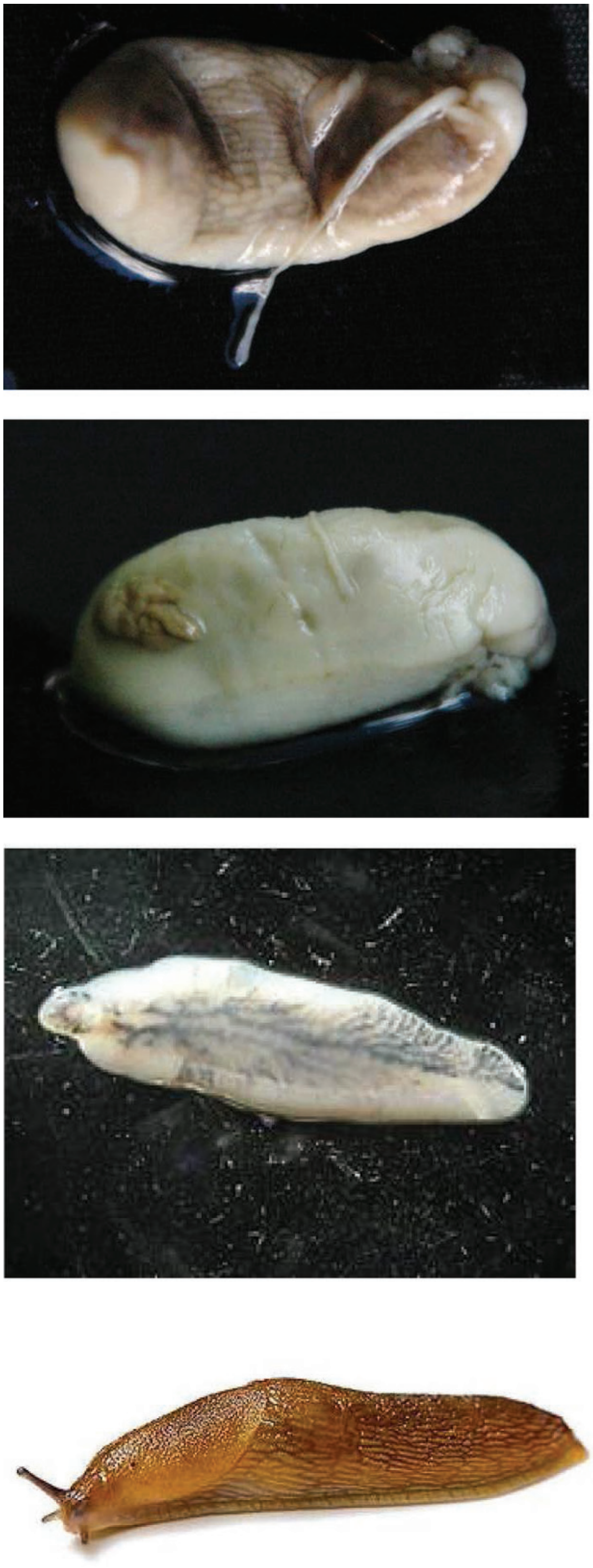


Figure 10.14 (Top) Slug recovered in stool; note the linear markings on the body. (Second) Underside of the slug. (Third) Fresh, living trematode; note the resemblance to the slug. (Bottom) Actual slug; note the markings on the body (this slug transmits *Angiostrongylus*). (Top and second, courtesy of Larry D. Gray, Clinical Microbiology Laboratory Consultants, LLC; bottom two images courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch10.f14

pathogenic protozoa is critical to good patient care. These cells should be reported and quantitated (rare, few, moderate, many). PMNs are frequently misidentified as a protozoan cysts, usually in the genus *Entamoeba*; as the PMNs begin to age, the lobed nucleus fragments into several parts, each resembling an amebic cyst nucleus. However, usually PMNs are seen in patients who tend to be symptomatic; patients with diarrhea do not tend to have protozoan cysts in the stool. The gut motility is so rapid that cyst formation does not occur; also, trophozoites do not encyst once they are outside of the body/gastrointestinal tract. The macrophage tends to have one large nucleus; the overall appearance can mimic the *Entamoeba* trophozoite.

Polymorphonuclear Leukocytes

Large numbers of PMNs are often found in patients with bacterial dysentery, and they may also be present in patients with intestinal amebiasis or ulcerative colitis (Fig. 10.19). These cells may be distinguished from *Entamoeba histolytica* as follows.

PMNs

1. Average size, 14 μm (10 to 12 μm on permanent stained smear)
2. Ratio of nuclear material to cytoplasm, 1:1
3. Nucleus: 2 to 4 segments connected by narrow, short chromatin bands. Segments may appear as separate nuclei like those of *E. histolytica*/*E. dispar* cysts—focus carefully to reveal connecting chromatin strands.
4. Granular cytoplasm
5. Trichrome staining characteristics similar to *E. histolytica*/*E. dispar*

E. histolytica/*E. dispar* (cysts)

1. Average size, 20 μm (less on permanent stained smear)
2. Ratio of nuclear material to cytoplasm, 1:10 to 1:12 (trophozoite), 1:2 to 1:3 (cyst)
3. Nucleus: round with central karyosome and peripheral chromatin
4. Uniform, agranular cytoplasm—may contain RBCs
5. Trichrome: green cytoplasm, dark red nuclear material

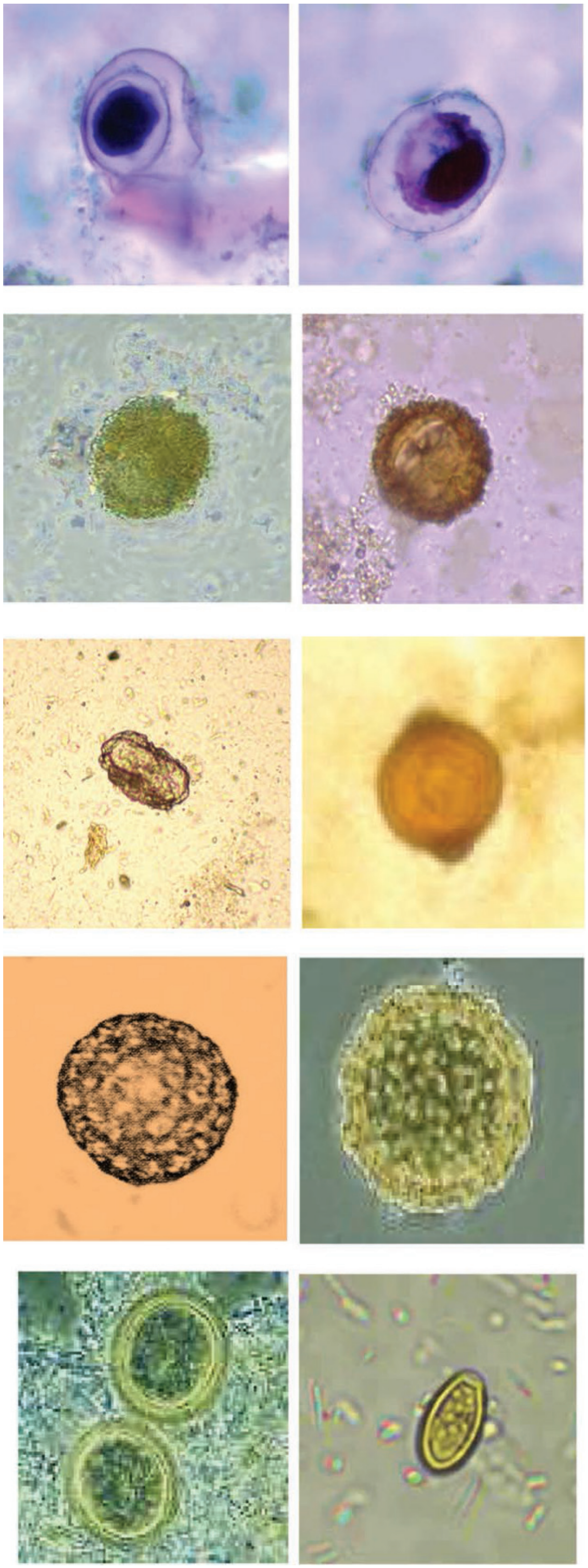


Figure 10.15 Various artifacts that may be seen in stool preparations (wet mounts or permanent stained smears). Many of these structures are pollen grains or egglike objects. Visually, they can be confused with some of the following helminth eggs: *Hymenolepis nana*, *Ascaris lumbricoides*, hookworm, and *Enterobius vermicularis*. doi:10.1128/9781555819002.ch10.f15

Eosinophils

The identification of eosinophils in a fecal specimen usually indicates the presence of an immune response in the host. This allergic response may be caused by a parasitic infection or by other antigens such as pollen or food. Eosinophils are essentially the same size as PMNs and are characterized by the presence of large, purple-staining granules (trichrome) and usually a bilobed nucleus, which may be obscured by the granules (Fig. 10.19). Eosinophils can also be seen in other specimens such as blood specimens and sputum specimens from asthmatic patients (10, 11). When reviewing blood smears for parasites, it is helpful to recognize the typical white blood cells as seen in Fig. 10.20. Unfortunately, not all microbiologists have had training in hematology per blood cell recognition, although in fecal specimens the white blood cells do not tend to have the precise morphology as that seen in routine blood films.

Macrophages

Macrophages (monocytes) are large, mononuclear, phagocytic cells that may resemble *E. histolytica/E. dispar* trophozoites (Fig. 10.19). These cells may be found in patients with intestinal amebiasis and ulcerative colitis and other inflammatory bowel diseases and can be differentiated from amebae as follows. One of the main differences is the ratio of nuclear material to cytoplasm, which is much larger in human cells.

Macrophages

1. Size: 30 to 60 µm, may be 5 to 10 µm less on permanent stained smear
2. Ratio of nuclear material to cytoplasm, 1:4–1:6
3. One large nucleus that may be irregular in shape (like monocyte nucleus)
4. Usually contains ingested debris, PMNs, and RBCs
5. May contain red-staining round bodies and nucleus may be absent
6. Trichrome staining characteristics similar to *E. histolytica/E. dispar*

E. histolytica/E. dispar (trophozoites)

1. Size: 12 to 60 µm; average, 20 µm (less on permanent stained smear)
2. Ratio of nuclear material to cytoplasm, 1:10 to 1:12

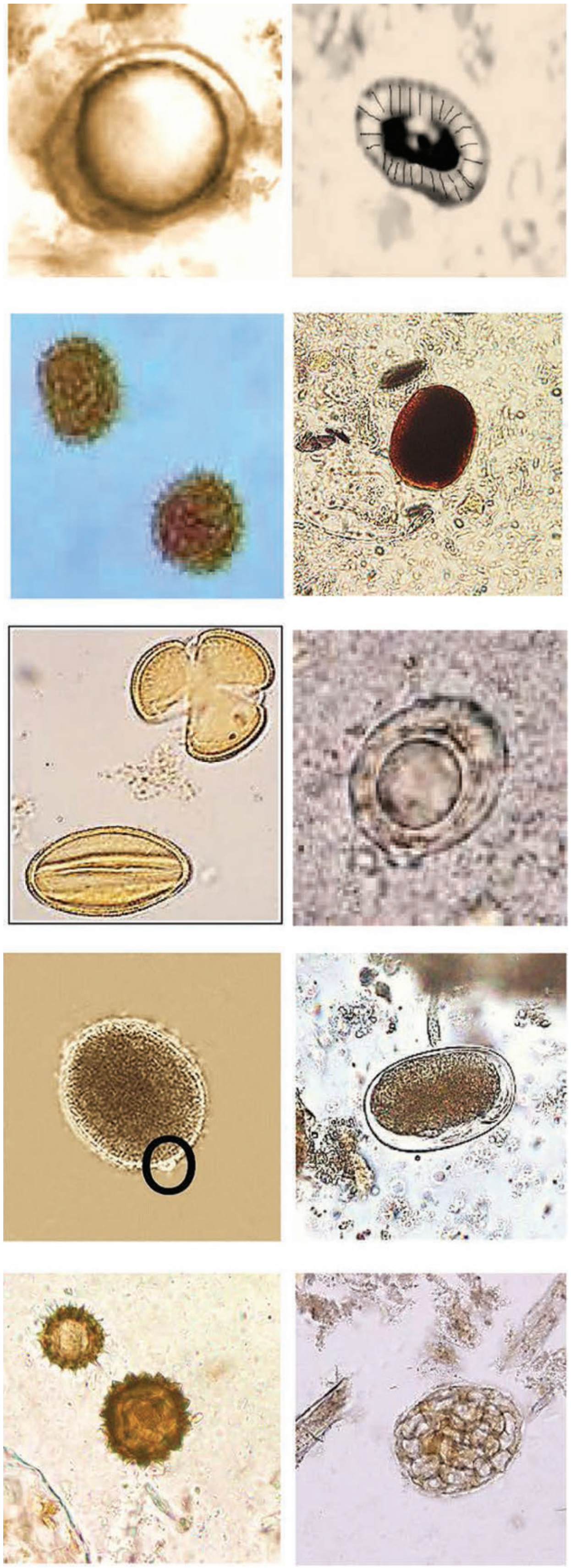


Figure 10.16 Various artifacts that may be seen in stool preparations (wet mounts). Note the egg-like structure in the fourth row (left). There is a small bubble (within the circle) that mimics the small knob found at the abopercular end of a *Diphyllobothrium latum* egg. doi:10.1128/9781555819002.ch10.f16

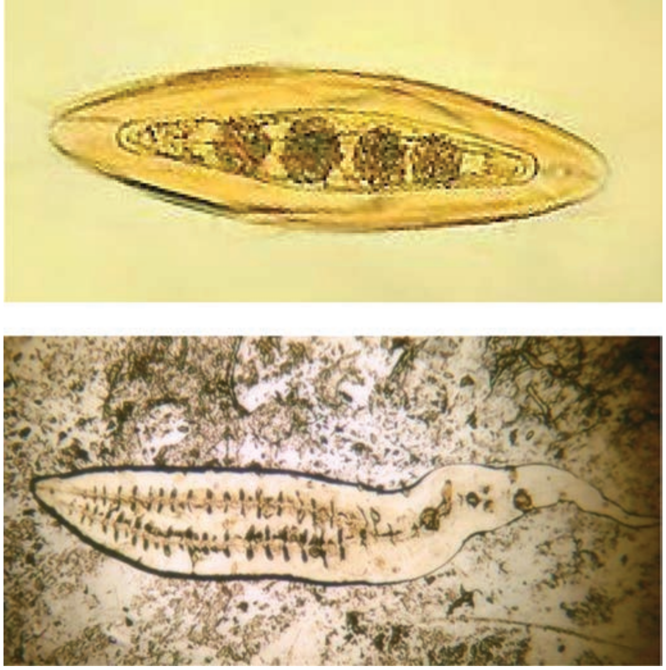


Figure 10.18 (Upper) "Beaver bodies," which are algae occasionally found in stool; (lower) leaf structure which resembles a trematode. (Courtesy of Joseph Dipersio.) doi:10.1128/9781555819002.ch10.f18

3. One nucleus, round, with central karyosome and peripheral chromatin
4. May contain RBCs and some debris; no PMNs
5. Nucleus always present
6. Trichrome: green cytoplasm, dark red nuclear material

Lymphocytes

Lymphocytes have a large, dense, dark-staining nucleus surrounded by very little cytoplasm. They are approximately two-thirds the size of PMNs (Fig. 10.20).

Red Blood Cells

In a buffy coat preparation, some RBCs may be present. These cells measure ~7.5 µm in a wet preparation and may be present in the stool as an indication of ulceration

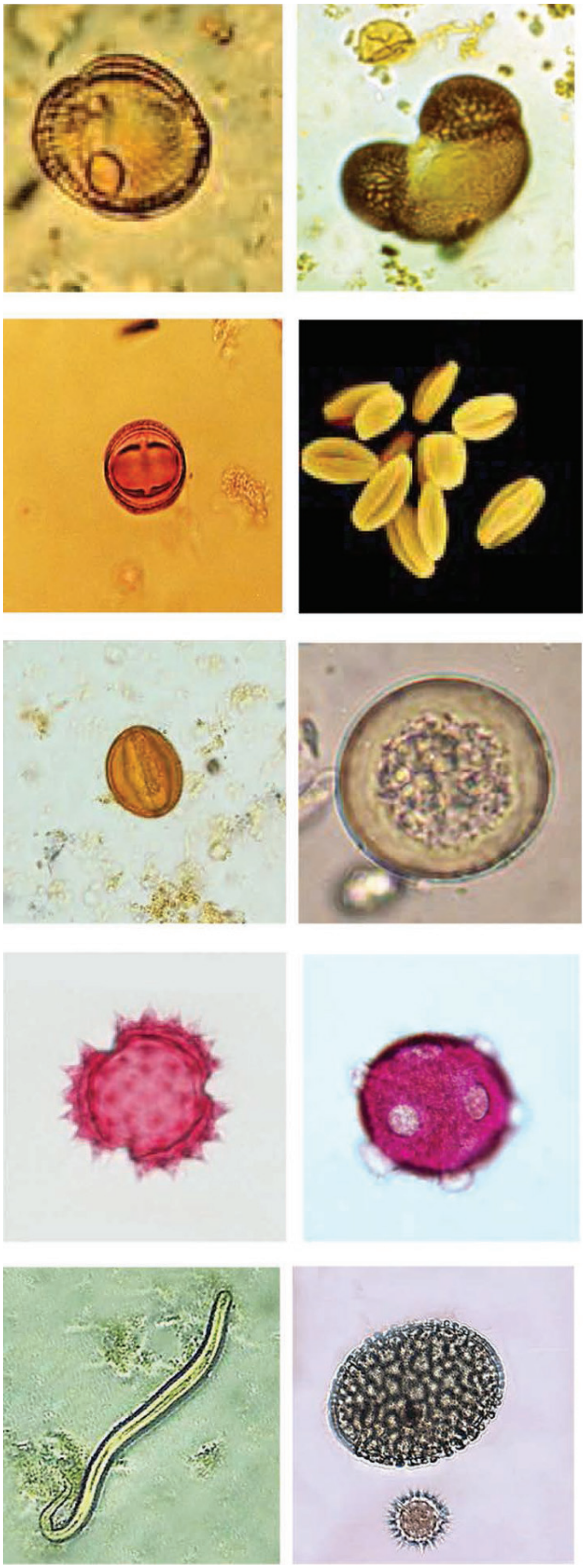


Figure 10.17 Various types of pollen grains and a root hair. These structures can mimic various helminth eggs (*Ascaris lumbricoides*, *Trichuris trichiura*), as well as nematode larvae. (Bottom row, left) Plant root hair (can mimic helminth larvae). (Row 4 from top: left, courtesy of Randy Oliver, photo by Gretchen D. Jones, United States Department of Agriculture; right, courtesy of http://cactiguide.com/forum/viewtopic.php?t=12480&view=next [accessed 7/23/13]). doi:10.1128/9781555819002.ch10.f17

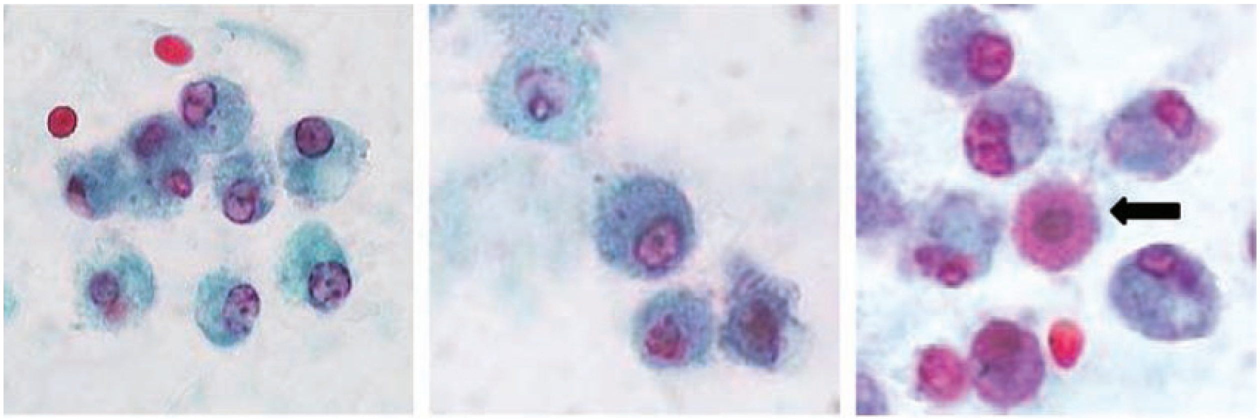
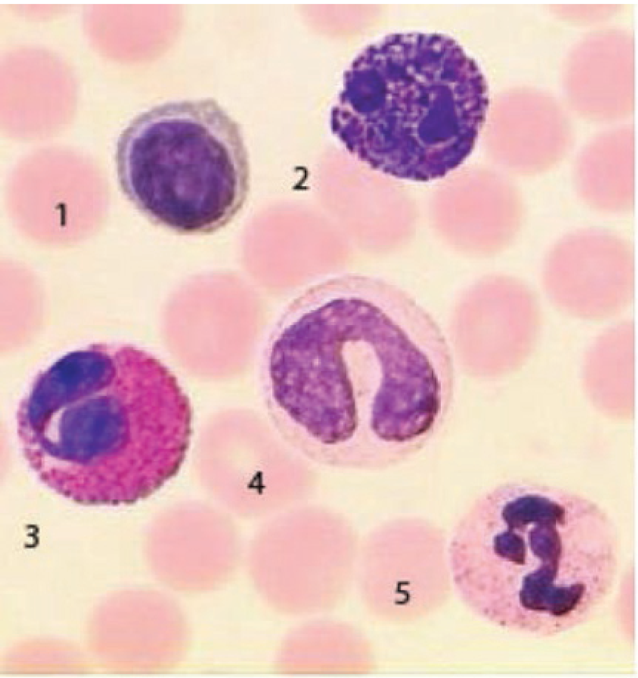


Figure 10.19 (Left) PMNs in a fecal specimen stained with trichrome stain. Note the lobed nuclei; if these cells have been in the stool for some time (unpreserved), the nuclei may fragment into four or five pieces, thus resembling multiple nuclei seen in amebic cysts. Note the two RBCs in the upper left. (Middle) Macrophages. Although these cells often resemble amebic trophozoites, the ratio of nuclear material to cytoplasm is quite different from that seen in actual protozoa (more nuclear material per cytoplasm in human cells). (Right) PMNs and macrophages; also note the eosinophil (arrow). doi:10.1128/9781555819002.ch10.f19

(parasitic or nonspecific) or other vascular or hemorrhagic problems. In the trichrome-stained slide, they appear as round or elongate (distortion may occur during smear preparation) red-purple bodies with no granules or inclusions and may be somewhat smaller than 7.5 μm (Fig. 10.19 and 10.20).

Figure 10.20 White blood cells in a stained blood film. (1) Lymphocyte. (2) Basophil. (3) Eosinophil. (4) Monocyte. (5) PMN. doi:10.1128/9781555819002.ch10.f20



Charcot-Leyden Crystals

Charcot-Leyden (CL) crystals are formed from the breakdown products of eosinophils and basophils and may be present in the stool or sputum with eosinophils or alone. They are slender crystals with pointed ends, and they stain red-purple with trichrome stain. Many different crystal sizes can be seen in the same specimen (Fig. 10.21). They indicate that an immune response has taken place, but the cause may or may not be parasitic. The presence of eosinophils and/or CL crystals in the stool may not correlate with an increased eosinophilia on the peripheral blood smear.

Identification of CL crystals in body fluids and secretions is considered an indicator of eosinophil-associated allergic inflammation. The overall structural fold of CL crystal protein is similar to that of galectins, and this is the first structure of an eosinophil protein to be determined (5). The protein exhibits weak carbohydrate binding activity for simple saccharides. There may be a potential intracellular and/or extracellular role(s) for the galectin-associated activities of CL crystal protein in eosinophil and basophil function in allergic diseases and inflammation (10).

Parasitic (primarily helminthic) infections push the immune system towards TH2 cytokine production and eosinophilia. Since eosinophil infiltration in infected organs and skin is a common finding, eosinophils are thought to have a specific role in parasite killing. Eosinophils have been considered as effectors of adaptive immune responses during parasitic infections and inflammatory processes. Their role in allergic and mucosal responses is mediated by membrane receptors that allow them to interact with IgE and IgA antibodies (10).

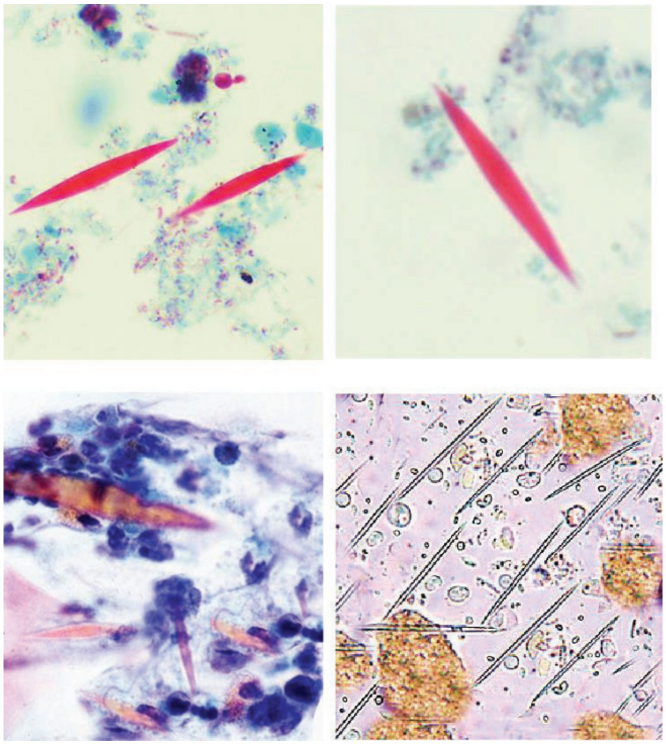


Figure 10.21 Charcot-Leyden (CL) crystals. These crystals are formed from the breakdown products of eosinophils and basophils and may be present in the stool, sputum, or other specimens with or without eosinophils. They tend to stain red to red-purple on the permanent stained fecal smears, often darker than nuclear material, and although the shape is consistent, there is a large size range in a single fecal smear or sputum mount. (Upper, left and right) CL crystals in trichrome-stained fecal smear. (Lower, left) CL crystals in a sputum specimen. (Lower, right) Pineapple crystals in stool. These may be confused with CL crystals; however, the pineapple crystals are much more slender.
doi:10.1128/9781555819002.ch10.f21

It has been reported that pineapple crystals sometimes mimic CL crystals in stool specimens. Apparently, these crystals can be found in fresh and canned pineapple and pineapple juice; also, they are not digested in the alimentary canal of humans. They range from 30 to 130 µm in length and 1 to 2 µm in width (Fig. 10.21). They appear to have parallel edges and are pointed at both ends. In wet preparations, they can resemble CL crystals. Those who routinely examine stool specimens for parasites should probably examine some pineapple juice under the microscope to become familiar with the appearance of these crystals.

Nonhuman Elements Seen in Feces (Yeast Cells)

There are many yeast cells that may be round to oval and measure ~4 to 8 µm which can be seen in fecal material. On a wet mount, they may resemble small protozoan cysts (*Endolimax nana* or *Entamoeba hartmanni*). After staining, they appear fairly uniform in color (red to green with trichrome stain) without many inclusions; if granules are seen, they are usually small but may resemble small protozoan karyosomes. Depending on the stain used, small yeast cells can be confused with coccidian oocysts or microsporidial spores. It is important to note the presence of budding yeast cells and/or pseudohyphae (clinically relevant only in freshly preserved specimens). The presence of branching pseudohyphae may be an indication of pathogenicity of the particular yeast present (usually *Candida* spp.) and should be reported. Large numbers of budding yeast cells in a fresh or freshly preserved specimen, indicating a potential source for a systemic infection, particularly in immunosuppressed patients, should also be reported (Fig. 10.22).

Note Because yeast can continue to grow if the stool is not immediately preserved, some laboratories do not report yeast, since the report can be misleading. They elect to call the physician and discuss the findings. Another option is to add a report comment indicating that reports of yeast (budding and/or pseudohyphae) might be misleading due to a lag time between stool passage and specimen fixation.

Insect Larvae

Finding insect larvae in stool is not common but may occur as a result of ingestion of whole larvae or adult insects with food. The presence of live larvae may suggest myiasis or, probably more common, contamination of the stool specimen. In these situations, it is always important to find out how and when the specimen was collected prior to submission, particularly if it was submitted as a fresh stool. Proper fixation of the suspected object is important for further identification (see chapter 9).

Spurious Infections

Spurious infections occur when individuals ingest liver from various animals. The various parasite eggs are digested free when the liver is eaten and will be passed in the stool for several days. Repeat ova and parasite examinations are recommended for several days to rule out a true infection. Examples are eggs of *Fasciola hepatica*, *Dicrocoelium dendriticum*, or *Capillaria hepatica*, which are present in the livers of cattle, sheep, and rodents, respectively (12). Occasionally, rarer eggs are found and may represent spurious infections acquired by eating the flesh of fish, birds, or other animals, both vertebrates and invertebrates (Fig. 10.23). In a true human infection with *Capillaria hepatica*, no eggs are found in the stool as they are in an infection with *Capillaria philippinensis*; diagnosis requires histologic examination. Eggs in liver biopsy speci-

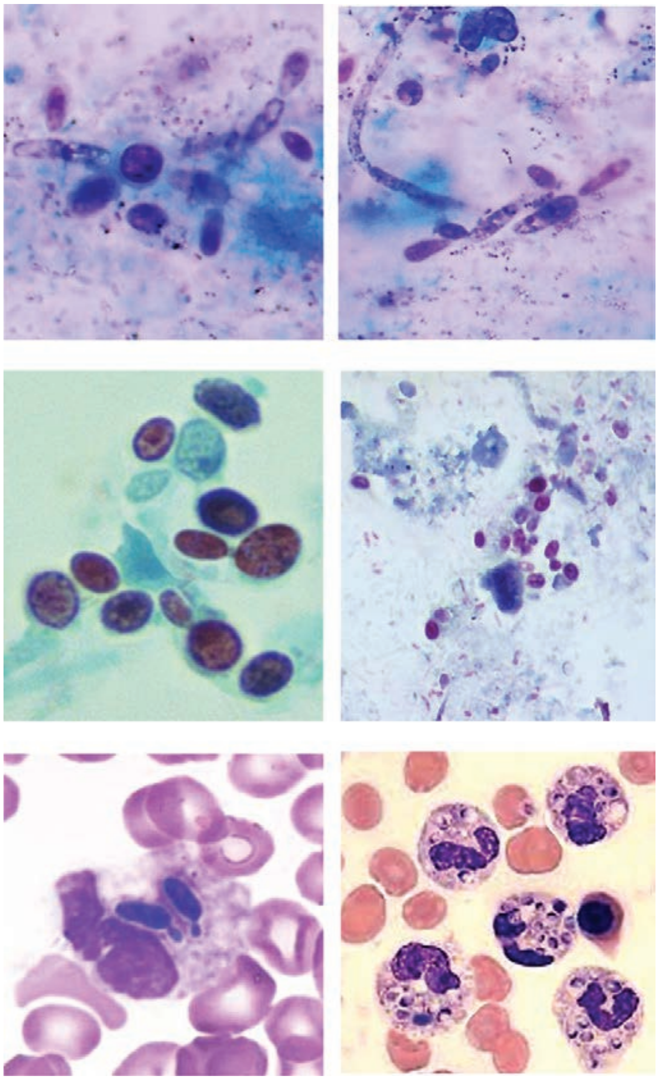


Figure 10.22 Yeast cells in clinical specimens. (Upper and middle) Trichrome-stained fecal smears. Depending on the size and permanent stain used (trichrome, modified acid-fast, modified trichrome), single yeast cells can often be confused with the coccidia or microsporidia. (Bottom left) Various yeasts in blood films; (right) *Histoplasma* within monocytes.
doi:10.1128/9781555819002.ch10.f22

mens can be identified on the basis of their characteristic morphology.

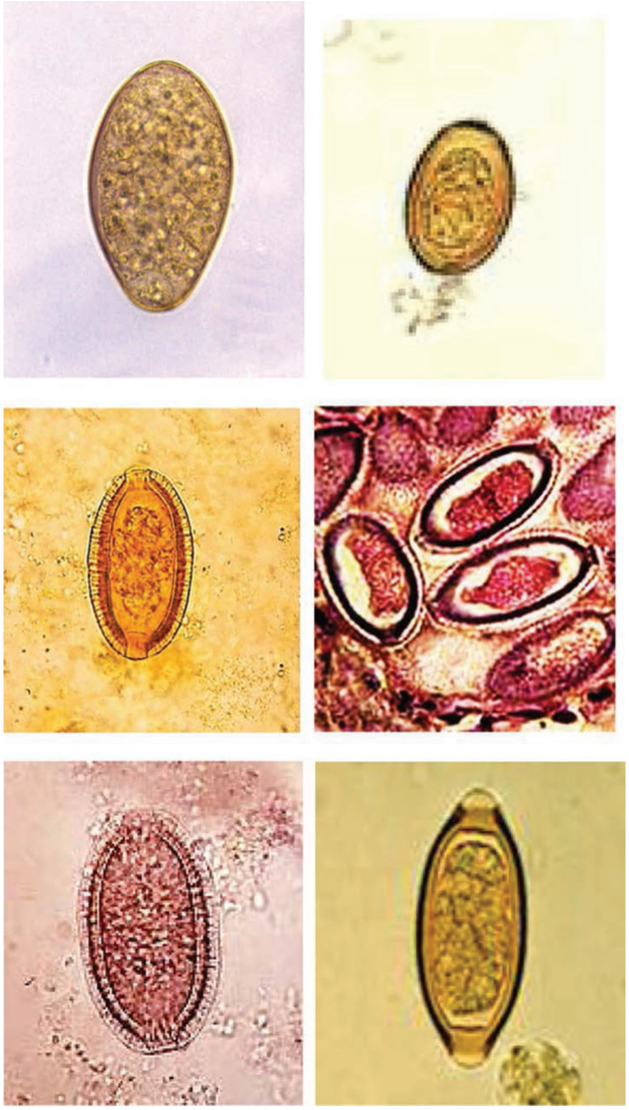


Figure 10.23 (Top row, left) *Fasciola hepatica* egg (130 to 150 µm by 63 to 90 µm) (image is lower magnification than *Dicrocoelium* egg); (right) *Dicrocoelium dendriticum* egg (38 to 45 µm by 22 to 30 µm). (Middle row, left) *Capillaria philippinensis* egg (51 to 68 µm by 30 to 35 µm), passed in the stool (resembles egg of *Trichuris trichiura*); (right) *Capillaria hepatica* eggs in liver. (Bottom row, left) *Capillaria philippinensis* egg; (right) *Trichuris trichiura* egg. Note the striated shell of *C. philippinensis* compared with the nonstriated shell seen in the *Trichuris* egg.
doi:10.1128/9781555819002.ch10.f23

Delusory Parasitosis (Delusional Infestation)

Occasionally, clinical specimens in which the patient has placed various objects or organisms to feign parasitism are submitted for examination. These patients are usually mentally disturbed and have often seen numerous physicians and submitted clinical specimens to many laboratories. The objects placed in the specimens range from pieces of thread to plant material to earthworms. Often, these patients call the laboratory with extensive histories of "parasitic infections" and seek referrals to other experts or consultants. They may bring photographs or samples of the "parasites" that they have found, all of which will be submitted for identification. These "infections" are not limited to specimens such as stool but may include skin, urine, and other samples for analysis. Often, these patients are women older than 50 years, with possible other medical problems, who present with a wide variety of symptoms. It is likely that dopaminergic and serotonergic dysfunction may play a role in delusional parasitosis; dopamine and serotonin antagonists may be relevant for the treatment of this disorder (13–16).

This disease can become a tremendous burden both for the patient and for the family. Patients can appear to be very rational about everything else, with the exception of their beliefs concerning infection or infestation with parasites. *However, actual infections have been misdiagnosed as delusory parasitosis when appropriate diagnostic procedures have not been used and the true infections have been missed.* A thorough investigation for parasites on the patient, on pets, and in the work and home environment should be completed before assigning a diagnosis of delusory parasitosis.

Patients with delusory parasitosis pose a difficult interdisciplinary problem for the medical system. Such patients avoid psychiatrists and consult dermatologists, microbiologists, or general practitioners but often lose faith in professional medicine. Epidemiology and history suggest that the imaginary pathogens may continually change, while the delusional aspect remains constant. Patients with self-diagnosed "Morgellons disease" can be seen as a variation of this delusional theme (15). Reference 15 provides very comprehensive information on all aspects of this disorder, including recommendations for physician/patient interactions and guidance algorithms.

The minimal criteria for delusional infestation are as follows:

- A strong conviction of being infested by pathogens (small, vivid, inanimate [rare], often "new to science") without any medical or microbiological evidence for this, ranging from overvalued ideas to a fixed, unshakable belief.
- Report of abnormal sensations in the skin explained by the first criterion.

Additional symptoms may include visual illusions or hallucinations. Although the "infestation" is generally associated with the skin, all parts of the body may be involved. Often this condition has gone on for months or years (15).

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11

Equipment, Supplies, Safety, and Quality System Recommendations for a Diagnostic Parasitology Laboratory: Factors Influencing Future Laboratory Practice

Equipment
Microscope
Centrifuge
Fume hood
Biological safety cabinet
Refrigerator-freezer
Supplies
Glassware
Miscellaneous supplies
ATCC quality control organisms
Safety: personnel and physical facilities
General precautions
Handwashing
Personal protective equipment (OSHA 2001 blood borne)
Handling specimens
Processing specimens
Spills
Disposal of contaminated materials
Standard precautions
Hepatitis exposure protocol
Dangerous properties of industrial materials
Current OSHA regulations for the use of formaldehyde
Latex allergy
Quality systems
Extent of services
Proficiency testing
In-house quality control
Patient outcome measures
Continuous quality improvement, total quality management, or 10-step and FOCUS-PDCA for performance improvement activities
CLIA '88 inspection process
New quality guidelines
ISO guidelines
CLSI (NCCLS) model
Factors influencing future laboratory practice
Managed care
Financial considerations
Current regulations
Decentralized testing
Laboratory services
Technological trends
Clinical decision support
Personnel issues
Changing demographics
Emerging diseases
Bioterrorism

Some of the following information has been adapted from *Clinical Microbiology Procedures Handbook*, published by the American Society for Microbiology (1). Additional information on the following topics can also be found in that publication. A number of excellent documents are also available for information and guidance (2–4).

Equipment

Microscope

Good, clean microscopes and light sources are mandatory for the examination of specimens for parasites (Fig. 11.1). Organism identification depends on morphologic differences, most of which must be seen under stereoscopic microscopes (magnification, ×50) or regular microscopes at low (×100), high dry (×400), and oil immersion (×1,000) magnifications. The use of a 50× or 60× oil immersion objective for scanning can be very helpful, particularly if the 50× oil and 100× oil immersion objectives are placed side by side. This arrangement on the microscope can help avoid accidentally getting oil on the 40× high dry objective. Calibration of the microscope is discussed later in this chapter. Although 5× oculars are acceptable, most laboratories select 10× oculars, preferably with a binocular, tilting head.

Types

Stereoscopic Microscope. A stereoscopic microscope is recommended for larger specimens (arthropods, tapeworm proglottids, and various artifacts). The total magnification usually varies from approximately ×10 to ×45, either with a zoom capacity or with fixed objectives (0.66×, 1.3×, and 3×) that can be used with 5× or 10× oculars. Depending on the density of the specimen or object being examined, you must be able to direct the light source either from under the stage or onto the top of the stage (may require a separate simple laboratory light directed at the top of the stage). Current uses include the examination of larger specimens (arthropods, artifact removed from clinical specimens, whole mounts, etc.).

doi:10.1128/9781555819002.ch11

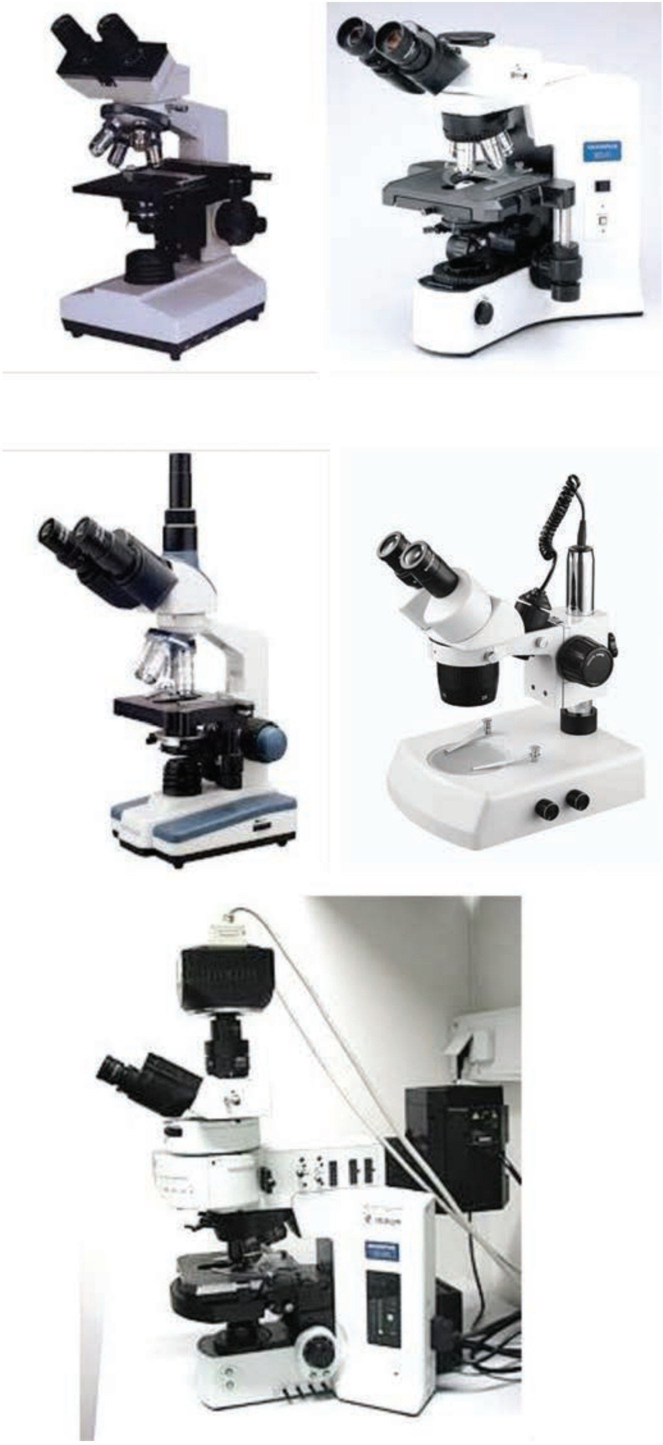


Figure 11.1 Microscopes routinely used in clinical laboratories. (Top row) Regular compound microscopes. (Second row, left) Microscope with tube for photography; (right) stereomicroscope. (Bottom) Fluorescence microscope.
doi:10.1128/9781555819002.ch11.f1

Regular Light Microscope. The light microscope should be equipped with the following:

1. *Head*. A binocular head is recommended and should be equipped with a diopter adjustment to compensate for focus variation in the eyes. A tilt head is highly recommended to accommodate various users.
2. *Oculars*. 10× oculars are required; 5× oculars can be helpful but are optional.
3. *Objectives*. 10× (low power), 40× (high power), 100× (oil immersion). Some laboratories are currently using 50× or 60× oil immersion lenses for screening permanent stained smears. Examination with a combination of the 50× oil or 60× oil and the 100× oil immersion lenses allows screening to proceed more quickly and eliminates the problem of accidentally getting oil on the high dry objective lens when switching back and forth between the 40× (high dry) and 100× (oil immersion) objectives.
4. *Stage*. A mechanical stage for both vertical and horizontal movement is required. Graduated stages are mandatory for recording the exact location of an organism on a permanent stained smear and recommended for any facility performing diagnostic parasitology procedures. This capability is essential for consultation and teaching responsibilities. *However, remember that calibration numbers for the exact location of an organism may not be the same for different microscopes and different stages.*
5. *Condenser*. A bright-field condenser equipped with an iris diaphragm is required; however, an adjustable condenser is not required with the newer microscopes. The condenser numerical aperture should be equal to or greater than the highest objective numerical aperture.
6. *Filters*. Both clear blue glass and white ground-glass filters are recommended.
7. *Light source*. The light source, along with an adjustable voltage regulator, is usually contained in the microscope base. This light source should be aligned as specified by the manufacturer. If the light source is external, the microscope must be equipped with an adjustable mirror and an adjustable condenser containing an iris diaphragm. The light source should be a 75- to 100-W bulb.
8. *Current Uses*. Routine microscopy in the microbiology laboratory, and parasitology in particular, includes the examination of direct wet mounts, concentration sediment and surface flotation layers, stained permanent smears, blood specimens, aspirates and small samples or smears/scrapings from any body site, and cytologic and histologic preparations (stained or unstained).

Fluorescence Microscope. The fluorescence microscope should be equipped with the following:

1. *Head*. A binocular head is recommended and should be equipped with a diopter adjustment to compensate for focus variation in the eyes. A tilt head is highly recommended to accommodate various users.
2. *Oculars*. 10× oculars are required; 5× oculars can be helpful but are optional.
3. *Objectives*. 10× (low power), 40× (high power), 100× (oil immersion).
4. *Stage*. A mechanical stage for both vertical and horizontal movement is required. Graduated stages are mandatory for recording the exact location of an organism on a permanent stained smear and recommended for any facility performing diagnostic parasitology procedures. This capability is essential for consultation and teaching responsibilities. *However, remember that calibration numbers for the exact location of an organism may not be the same for different microscopes and different stages*.
5. *Light source*. Mercury-free microscopy (MFM) provides a new approach that encourages the selection of modern mercury-free light sources to replace more traditional mercury-based arc lamps. Microscope performance is enhanced with new solid-state technologies; they offer a more stable light intensity output and have a more uniform light output across the visible spectrum. Solid-state sources eliminate mercury, eliminate the cost of consumable bulbs (lifetime ~200 hours), use less energy, reduce the instrument downtime when bulbs fail, and reduce the staff time required to replace and align bulbs. With lifetimes of approximately tens of thousands of hours, solid-state replacements can pay for themselves over their lifetime, are sustainable, and comply with institutional and government body mandates to reduce energy consumption, carbon footprints, and hazardous waste. Solid-state light-emitting diode (LED)-based light sources have been used in the laboratory for some time. However, advancements in solid-state "light engine" technology now provide laboratories with mercury alternatives that are straightforward to operate, environmentally friendly, affordable to buy, and better suited for fluorescence microscopy.
6. *Fluorescence filter set*. Usually housed in cube-shaped optical blocks, fluorescence filter sets include excitation and barrier (emission) filters along with a dichromatic mirror. The filter block is positioned in the vertical illuminator, above the objective, so that incident illumination can be directed through the excitation filter and reflected from the surface of the dichromatic mirror onto the specimen. The fluorescence emitted by the specimen is gathered by the objective and transmitted through the dichromatic mirror to the eyepieces or detector system.
7. *Quenching*. Quenching refers to any process which decreases the fluorescence intensity of a given substance. Although it is important to store slides in the dark (*Giardia*/*Cryptosporidium* FA reagent slides) during incubation and prior to reading, personal use indicates quenching is very minimal; slides may retain fluorescence for several days to weeks.
8. *Current uses*. There are a number of diagnostic procedures available that rely on fluorescence. Examples include fecal immunoassays for antigen detection (FA) for *Cryptosporidium*, *Giardia*, and microsporidia (not FDA approved); demonstration of autofluorescence with *Cyclospora*; various stains such as calcofluor white, auramine, rhodamine; various serologic methods for antibody detection; and research products for labeling/conjugation/linking various components.

Maintenance (5)

1. Use a camel hair brush to remove dust from all optical surfaces; remove oil and finger marks immediately from the lenses with *several thicknesses* of lens tissue. *Single-thickness lens tissue may permit corrosive acids from the fingers to damage the lens. Do not use any type of tissue other than lens tissue; otherwise you may scratch the lens. Use very little pressure, to prevent removal of the coatings on external surfaces of the lenses*.
2. Use water-based cleaning solutions for normal cleaning. If you have to use organic solvents, use them in very small amounts and only if absolutely necessary to remove oil from the lens. *Since microscope manufacturers do not agree on solvents to be used, each company's recommendations should be consulted*. One recommended solvent is 1,1,1-trichloroethane; it is good for removing immersion oil and mounting media and does not soften the lens sealers and cements. *Xylene, any alcohols, acetone, or any other ketones should never be used as cleaning fluids*.
3. After the lamp has been installed into the lamp holder, clean it with lens tissue moistened in 70% isopropyl or ethyl alcohol (to remove oil from fingers). *Make sure that the lamp is cool and the switch is in the off position when replacing or removing the lamp*.

4. Clean the stage with a small amount of disinfectant (70% isopropyl or ethyl alcohol) when it becomes contaminated.
5. Using petroleum jelly or light grease, clean and lubricate the substage condenser slide as needed.
6. Cover the microscope when not in use. In extremely humid climates (a relative humidity of more than 50%), good ventilation is necessary to prevent fungal growth on the optical elements.
7. At least annually, schedule a complete general cleaning and readjustment to be performed by a factory-trained and authorized individual. If microscopes are in continual use, maintenance should be performed twice a year. Record all preventive maintenance and repair data (date, microscope identification number, names of company and representative, maintenance and/or repairs, part replacement, recommendations for next evaluation, estimated cost if you have such information). This information should be cumulative so that a review for each piece of equipment can be scanned quickly for continuing problems, justification for replacement requests, etc. Depending on the physical site and use of the microscope, laboratories may use different maintenance schedules.

Calibration

The identification of protozoa and other parasites depends on several factors, one of which is size. Any laboratory doing diagnostic work in parasitology should have a calibrated microscope available for precise measurements (6). Measurements are made with a micrometer disk that is placed in the ocular of the microscope; the disk is usually calibrated as a line divided into 50 units (Fig. 11.2). Depending on the objective magnification used, the divisions in the disk represent different measurements. The ocular disk division must be compared with a known calibrated scale, usually a stage micrometer with a scale of 0.1- and 0.01-mm divisions. Although there is not universal agreement, it is probably appropriate to recalibrate the microscope once or more each year. This recommendation should be followed if the microscope has received heavy use or has been bumped or moved multiple times. Often, the measurement of red blood cells (approximately 7.5 µm) is used to check the calibrations of the three magnifications (×100, ×400, and ×1,000). Latex or polystyrene beads of a standardized diameter (Sigma, J. T. Baker, etc.) can be used to check the calculations and measurements. Beads of 10 and 90 µm in diameter are recommended.

Supplies

1. Ocular micrometer disk (line divided into 50 units) (any laboratory supply distributor [Fisher, Baxter, Scientific Products, VWR, etc.])

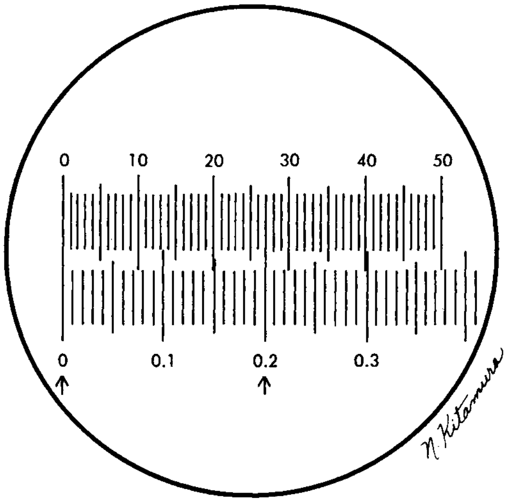


Figure 11.2 Ocular micrometer, top scale; stage micrometer, bottom scale. (Illustration by Nobuko Kitamura; modified from references 1, 6, and 55.) doi:10.1128/9781555819002.ch11.f2

2. Stage micrometer with a scale of 0.1- and 0.01-mm divisions (Fisher, Baxter, Scientific Products, VWR, etc.)
3. Immersion oil
4. Lens paper (**do not use other types of tissues**)
5. Binocular microscope with 10×, 40×, and 100× objectives. Other objective magnifications may also be used (50× oil or 60× oil immersion lenses).
6. Oculars of 10×. Some may prefer 5×; however, smaller magnification may make final identifications more difficult.
7. Single 10x ocular to be used to calibrate all laboratory microscopes (to be used when any organism is being measured – must be used on each calibrated microscope (oculars are NOT interchangeable).

Note All measurements should be documented in quality control (QC) records.

Procedure

1. Unscrew the eye lens of a 10× ocular, and place the micrometer disk (engraved side down) within the ocular. Use lens paper (several thicknesses) to handle the disk; keep all surfaces free of dust and lint.
2. Place the calibrated micrometer on the stage, and focus on the scale. You should be able to distinguish the difference between the 0.1- and 0.01-mm divisions. Make sure that you understand the divisions on the scale before proceeding.
3. Adjust the stage micrometer so that the "0" line on the ocular micrometer is exactly lined up on top of the 0 line on the stage micrometer.

4. After these two 0 lines are lined up, do not move the stage micrometer any farther. Look to the right of the 0 lines for another set of lines that is superimposed. The second set of lines should be as far to the right of the 0 lines as possible; however, the distance varies with the objectives being used (Fig. 11.2).
5. Count the number of ocular divisions between the 0 lines and the point where the second set of lines is superimposed. Then, on the stage micrometer, count the number of 0.1-mm divisions between the 0 lines and the second set of superimposed lines.
6. Calculate the portion of a millimeter that is measured by a single small ocular unit.
7. When the high dry and oil immersion objectives are used, the 0 line of the stage micrometer will increase in size whereas the ocular 0 line will remain the same size. The thin ocular 0 line should be lined up in the center or at one edge of the broad stage micrometer 0 line. Thus, when the second set of superimposed lines is found, the thin ocular line should be lined up in the center or at the corresponding edge of the broad stage micrometer line.

   Examples:

   A. $$\frac{\text{Stage reading}}{\text{ocular reading}} \times \frac{1{,}000\ \mu\text{m}}{1\ \text{mm}} = \text{ocular units } (\mu\text{m})$$

   B. Low power (10×)
   $$\frac{0.8\ \text{mm}}{100\ \text{units}} \times \frac{1{,}000\ \mu\text{m}}{1\ \text{mm}} = 8.0\ \mu\text{m (factor)}$$

   C. High dry power (40×)
   $$\frac{0.1\ \text{mm}}{50\ \text{units}} \times \frac{1{,}000\ \mu\text{m}}{1\ \text{mm}} = 2.0\ \mu\text{m (factor)}$$

   D. Oil immersion (40×)
   $$\frac{0.05\ \text{mm}}{62\ \text{units}} \times \frac{1{,}000\ \mu\text{m}}{1\ \text{mm}} = 0.8\ \mu\text{m (factor)}$$

Example: If a helminth egg measures 15 ocular units by 7 ocular units (high dry objective), using the factor of 2.0 µm for the 40× objective (example C above), the egg measures 30 by 14 µm and is probably *Clonorchis sinensis*.

Example: If a protozoan cyst measures 23 ocular units (oil immersion objective), using the factor of 0.8 µm for the 100× objective (example D above), the cyst measures 18.4 µm.

Results. For each objective magnification, a factor will be generated (1 ocular unit = certain number of micrometers). If standardized latex or polystyrene beads or a red blood cell is measured with various objectives, the size of the object measured should be the same (or very close), regardless of the objective magnification. The multiplication factor for each objective should be posted (either on the base of the microscope or on a nearby wall or bulletin board) for easy reference. Once the number of ocular lines per width and length of the organism is measured, then, depending on the objective magnification, the factor (1 ocular unit = certain number of micrometers) can be applied to the number of lines to obtain the width and length of the organism. Comparison of these measurements with reference measurements in various books and manuals should confirm the organism identification.

Procedure Notes for Microscope Calibration

1. The final multiplication factors will only be as good as your visual comparison of the ocular 0 and stage micrometer 0 lines.
2. As a rule of thumb, the high dry objective (40×) factor should be approximately 2.5 times more than the factor obtained from the oil immersion objective (100×). The low-power objective (10×) factor should be approximately 10 times that of the oil immersion objective (100×).

Limitations of Microscope Calibration

1. After each objective has been calibrated, the oculars containing the disk and/or the objectives cannot be interchanged with corresponding objectives or oculars on another microscope.
2. Each microscope used to measure organisms must be calibrated as a unit. The original oculars and objectives that were used to calibrate the microscope must also be used when an organism is measured.
3. The objective containing the ocular micrometer can be stored until needed. This single ocular can be inserted when measurements are taken. However, this particular ocular containing the ocular micrometer disk must have also been used as the ocular during microscope calibration.

Centrifuge

A table or floor model centrifuge to accommodate 15-ml centrifuge tubes is recommended. It is also helpful to have a centrifuge that can hold 50-ml centrifuge tubes, particularly when some of the commercial concentration systems are used. Regardless of the model, a free-swinging or horizontal head is recommended. With this type of centrifuge, the sediment is deposited evenly on the bottom of the tube and the flat surface of the sediment allows removal of the supernatant fluid from the sediment, particularly when you cannot turn the tube upside down to pour out the supernatant fluid. Most laboratories are using carrier cups that have screw-cap closures; this feature, in addition to capped centrifuge tubes, will minimize any aerosol formation and/or distribution.

Maintenance (5)

1. Before each run, visually check the carrier cups, trunnions, and rotor for corrosion and cracks. If anything is found to be defective, replace it immediately or remove the equipment from service. Check for the presence and insertion of the proper cup cushions before each run.
2. At least quarterly, check the speed at all regularly used speeds with a stroboscopic light to verify the accuracy of a built-in tachometer or speed settings. Remember to record results. Some laboratories perform this function every 6 months or yearly.
3. Following a breakage or spill and at least monthly, disinfect the centrifuge bowl, buckets, trunnions, and rotor with 10% household bleach or phenolic solution. Following disinfection, rinse the parts with warm water and perform a final rinse with distilled water. Thoroughly dry the parts with a clean absorbent towel to prevent corrosion. At least quarterly, brush the inside of the cups with mild warm soapy water and use fine steel wool to remove deposits; the cups should then be rinsed in distilled water and thoroughly dried.
4. Follow manufacturer's recommendations for preventive maintenance (lubrication).
5. Semiannually, check brushes and replace if worn to 1/4 in. (1 in. = 2.54 cm) of the spring. Also semiannually, check the autotransformer brush and replace if worn to 1/4 in. of the spring.
6. Record all information relating to preventive maintenance and repair (date, centrifuge identification number, names of company and representative, maintenance and/or repairs, part replacement, recommendations for next evaluation, estimated cost if you have such information). This information should be cumulative so that a review for each piece of equipment can be scanned quickly for continuing problems, justification for replacement requests, etc.

Fume Hood

Chemical fume hoods should be used when there is risk of exposure to hazardous fumes or splashes while preparing or dispensing chemical solutions. Airflow is generally controlled by a movable sash and should be in the range of 80 to 120 ft/min (1 ft = 30.48 cm). Chemical fume hoods are certified annually. Although a fume hood is not required for diagnostic parasitology work, many facilities keep the staining setup and formalin (see below for a discussion of regulations regarding the use of formaldehyde) in a fume hood. Fume hoods may also be preferred for the elimination of odors. The placement of reagents, supplies, and equipment within the hood should not interfere with the proper airflow.

Maintenance

1. At least yearly, with the sash fully open and the cabinet empty, check the air velocity with a thermoanemometer (minimum acceptable face velocity, 100 ft/min) (7). Also, a smoke containment test should be performed with the cabinet empty to verify proper directional face velocity.
2. Lubricate the sash guides as needed.

Biological Safety Cabinet

The Class II-A1 or II-A2 biological safety cabinet (BSC) is best suited and recommended for the diagnostic laboratory. BSCs operate at a negative air pressure with air passing through a HEPA filter, and the vertical airflow serves as a barrier between the cabinet and the user. Although a BSC is not required for processing routine specimens in a diagnostic parasitology laboratory, some laboratories use class I (open-face) or class II (laminar-flow) BSCs for processing all unpreserved specimens (3). Use of a BSC is recommended if the laboratory is performing cultures for parasite isolation. However, remember that BSCs should not be used as fume hoods. Toxic, radioactive, or flammable vapors or gases are not removed by HEPA filters (8).

Maintenance (5)

1. After each use, disinfect the work area. Since UV radiation has very limited penetrating power, do not depend on UV irradiation to decontaminate the work surface (9). At least weekly, clean UV lamps (in the off position) with 70% isopropyl or ethyl alcohol.
2. At least annually, have class I BSCs certified. They should also be certified after installation but before use and after they have been relocated or moved. Certification should include the following and will be documented by the trained company representative (contracted to handle the BSC inspection).
   A. Measurements of the air velocity are taken at the midpoint height approximately 1 in. behind the front opening. Measurements should be made approximately every 6 in.
   B. The average face velocity should be at least 75 linear ft/min. A thermoanemometer with a sensitivity of ±2 linear ft/min should be used (10).

   C. With the cabinet containing the routine work items, such as a Bunsen burner, test tube rack, bacteriological loop and holder, etc., a smoke containment test should be performed to determine the proper directional velocity.
   D. Record the date of recertification, the names of the individual and company recertifying the cabinet, and any recommendations for future service. Any maintenance performed should also be documented in writing.
3. Replace the filters as needed.
4. On installation, have a class II BSC certified to meet Standard 49 of the National Sanitation Foundation, Ann Arbor, MI (10). The cabinet must also be recertified at least annually and/or when it is moved, after filters are replaced, when the exhaust motor is repaired or replaced, and when any gaskets are removed or replaced. Record the date of recertification, the names of the individual and company performing the service, and any recommendations for future service.

Refrigerator-Freezer

Any general-purpose laboratory (non-explosion-proof) or household-type refrigerator-freezer (4 to 6°C) can be used in the parasitology laboratory. Solvents with flash points below refrigeration temperature should not be stored, even in modified (explosion-proof) refrigerators.

Maintenance

1. On a daily basis, monitor and record the temperature of the refrigerator. The thermometer should be placed into a liquid to permit stable temperature recording, or thermocouples may be used.
2. On a daily basis, monitor and record the temperature of the freezer. The thermometer should be placed in antifreeze (any brand with freezing point below that of the freezer, e.g., ethylene-glycol-water solutions, glycerol-water solutions, or Prestone) to permit stable temperature recording. Thermocouples may be used instead.
3. Periodically when the door is opened, check to see if the fan is operational.
4. Monthly, check the door gasket for deterioration, cracks, and proper seal. Seal problems are often seen when ice begins to build up in a freezer or the temperature is not holding. Periodically, petroleum jelly can be rubbed onto the door gasket to lubricate the material and to help maintain flexibility for a tight seal when the door is shut.
5. Semiannually, clean the condenser tubing and air grill with a vacuum cleaner.
6. Semiannually, check to ensure that the drain tubes are kept open.
7. Annually, wash the interior with a warm solution of baking soda and water (approximately 1 tablespoon/qt [ca. 13 to 14 g/0.946 liter]). Rinse with clean water, and dry. Also, wash the door gasket and water collection tray with a mild soap and water. If the gasket accumulates a black mold, scrub with 50% household bleach solution and a small brush. Rinse with clean water, and dry.

Supplies

Supplies for the diagnostic parasitology laboratory are often identical to those needed for routine work in other areas of microbiology. Although not every size of glassware used is specified, the list below should be helpful for anyone setting up a laboratory for this type of work.

Glassware

1. Disposable glass or plastic pipettes and bulbs (some sterile for culture work)
2. Pipettes: 1, 5, and 10 ml (some sterile for culture work)
3. Glass slides (1 by 3 in., or larger if preferred). Slides with rounded edges are now available (safety-“sharps”).
4. Coverslips (22 by 22 mm; no. 1 or larger if preferred)
5. Beakers, 250, 500, 1,000, and 2,000 ml
6. Covered Coplin jars or staining dishes (with slide rack)
7. Graduated cylinders, 50, 100, 500, and 1,000 ml
8. Mortar and pestle (range of sizes)
9. Flasks, Erlenmeyer, 500 and 1,000 ml
10. Flasks, volumetric, 500 and 1,000 ml
11. Bottles, brown, 150 to 200 ml
12. Bottles, clear, 100, 500, and 1,000 ml
13. Bottles, airtight, 50 ml
14. Funnel (glass) to hold filter paper
15. Büchner funnel
16. Centrifuge tubes, 15 and 50 ml (some with screw caps)
17. Petri dish, plastic, sterile
18. Tubes, screw-cap, 13 by 100 mm or 16 by 125 mm (some sterile for culture work)
19. Plastic syringe, 15 ml
20. Sterile syringes (glass or plastic), 1, 10, 20, and 50 ml

Miscellaneous Supplies

1. Culture tube racks
2. Gauze (woven or pressed)
3. Applicator sticks (wood)

4. Sterile cryovials or screw-cap vials (to hold 1 ml)
5. Box for vial storage in freezer
6. Filter paper, Whatman no. 1 and Whatman no. 42
7. Sterile filtration system
8. Membrane filters (pore size, 0.22 μm) (to be used with sterile filter)
9. Nuclepore membrane filter, 25-mm, 5-μm, and 3-μm porosity
10. Swinney filter adapter (attaches to syringe, holds filter)
11. Filter paper pad, 25 mm (used to support the membrane filter in the Swinney adapter)
12. Bacteriological loop
13. Sterile syringe needles, 20 and 27 gauge
14. Vaspar
15. Parafilm (American Can Co.) or equivalent
16. Slide boxes for positive-slide storage
17. Forceps and scissors
18. Stage micrometer with scale of 0.1- and 0.01-mm divisions
19. Disk micrometer divided into 50 units
20. Biohazard container with disinfectant for proper disposal of slides, tubes, and pipettes
21. Biohazard container for proper disposal of patient specimens
22. Microscope lens paper

ATCC Quality Control Organisms

1. An 18- to 24-h-old culture of *Escherichia coli* or *Enterobacter aerogenes*
2. ATCC 30010 (*Acanthamoeba castellanii*)
3. ATCC 30133 (*Naegleria gruberi*)
4. ATCC 30925 (*Entamoeba histolytica* HU-1:CDC)
5. ATCC 30015 (*Entamoeba histolytica* HK-9)
6. ATCC 30001 (*Trichomonas vaginalis*)
7. ATCC 30883 (*Leishmania mexicana*)
8. ATCC 30160 (*Trypanosoma cruzi*)

Note None, some, or all of these QC organisms will be required, depending on the culture procedures performed in the laboratory.

Safety: Personnel and Physical Facilities

General Precautions (1–22)

1. *Be careful!* All material to be received by or discarded from the laboratory must be considered potentially pathogenic.
2. Smoking, eating, or drinking in the laboratory is not permitted.
3. Do not work with uncovered open cuts or broken skin. Cover them with a Band-Aid, finger cot, or other suitable means, such as rubber or plastic gloves.
4. Mouth pipetting of specimens is not permitted.
5. Do not create aerosols. Remember, infectious diseases, such as infectious hepatitis, may be transmitted by aerosols produced by centrifuges, stirrers, pipettors, etc. Exercise extreme care when using such equipment. Cool inoculating loops or needles before touching colonies on plates or inserting into liquid material.
6. Develop the habit of keeping your hands away from your mouth, nose, and eyes. Wash hands well with soap before leaving the laboratory.
7. Do not lay personal articles, such as eyeglasses, on the bench in your work area.
8. Laboratory coats or gowns are not to be worn outside the laboratory, particularly not to the employee lounge or cafeteria or out of the building.
9. Wipe off benches in your working area with disinfectant *before and after* each day's work. Keep your area clean at all times.
10. In case of injury or unusual incident, however slight, the supervisor in charge must be immediately notified, and a report to occupational health facility is required. Also fill out an accident report form. Major accidents must be documented and reported in detail to the supervisor and chief of microbiology. The report should indicate:
    A. Cause of the accident
    B. Type of contamination or hazard
    C. List of personnel possibly exposed and amount of exposure to possible pathogenic material
    D. Decontamination procedures taken if pathogenic material was involved
    E. Actions taken to prevent recurrence
    F. Actions taken to safeguard or monitor employees
11. Infections may be spread by a number of routes in the laboratory. The actual occurrence of an infection depends on the virulence of the infecting agent, susceptibility of the host, route of entry, inoculating dose, etc. (Table 11.1).
    A. *Airborne*. Droplets and aerosols may be formed by simply removing caps or cotton plugs or swabs from tubes. Heating liquids on needles too rapidly may also create an aerosol. Breakages in centrifuges are serious accidents (Table 11.2).

TABLE 11.1 Potential exposures to laboratory-acquired parasitic infections (2, 3)

Parenteral or aerosolization

- Recapping a needle
- Removing a needle from the syringe
- Leaving a needle on the counter, point up
- Dropping a syringe
- Breaking hematocrit tube while pressing the end into clay
- Performing venipuncture on agitated patient
- Sudden animal movement during an inoculation procedure
- Creation of aerosols during tapeworm proglottid injection
- Creation of aerosols while working with cultures (bacteria, viruses, fungi, blood parasites, free-living amebae)
- Preparation of touch or squash smears from patient tissue
- Spill cleanup

Animal or vector bites

- Bitten by an infected animal (e.g., mouse or hamster)
- Bitten by infected mosquito or tick (e.g., mosquito colony)

Skin or mucous membrane exposure

- Not wearing gloves during procedure; using torn or damaged gloves
- Failure to wear laboratory coat (closed sleeves, closed front over clothes)
- Accidentally touching face or eyes during handling of infectious materials
- Exposure of eyes, nose, or mouth to potential aerosols
- Waste disposal
- Working on contaminated surfaces
- Handling contaminated equipment
- Manipulation of contact lenses

Ingestion/oral

- Mouth pipetting
- Sprayed with inoculum droplets from coughing or regurgitating animal
- Contaminated articles or fingers placed in the mouth
- Eating or drinking within the working laboratory space
- Applying lipstick or lip balm

Other reasons for potential exposures

- Working in disorganized laboratory bench setting
- Working too fast
- Failure to receive proper training
- Assumption that agent is not infectious to humans
- Assumption that agent(s) are no longer viable
- Using defective equipment
- Accidental spills while subculturing organisms

TABLE 11.2 Laboratory functions that may produce aerosols

Laboratory function	Practice or potential cause (type of relevant culture)
Inoculating-loop manipulations	Subculturing or streaking culture plates
	"Cooling" a loop in culture medium
	Flaming a loop
	(Free-living ameba culture systems)
Pipette	Mixing microbiological suspensions or fresh specimens
	Pipette spills on hard surfaces and benches
	Blowing contents from pipettes
	(Any function involving pipettes and liquids)
Needle and syringe manipulation	Expelling air
	Withdrawing needle from stopper
	Injecting animals
	Spray created when needle separates from syringe
	(Any function involving syringe-needle combination)
Other laboratory functions	Centrifugation with open containers (centrifuge tubes and/or carriers)
	Blenders, shakers, sonicators, and mixing instruments (specimens, cultures)
	Pouring or decanting fluids (specimen processing)
	Opening culture containers (any parasitic culture)
	Spillage of infectious material
	Lyophilization and filtration under vacuum

B. *Ingestion*. Ingestion can occur through mouth pipetting, failure to wash hands after handling specimens or cultures, or smoking, eating, and drinking in the laboratory.

C. *Direct inoculation*. Scratches, needlesticks, cuts from broken glass, or animal bites may permit direct inoculation.

D. *Skin contact*. Some very virulent organisms, and others not so virulent, can enter through small cuts or scratches or through the conjunctiva of the eye.

E. *Vectors*. Flies, mosquitoes, ticks, fleas, and other ectoparasites can be potential sources of infection in the laboratory, especially if animal work is performed.

12. Personnel who display risk-prone behavior or are pregnant, immunocompromised, or immunosuppressed should be restricted from performing work with highly infectious microorganisms and, in some situations, be restricted to a low-risk laboratory (22).

Handwashing

Handwashing is the most important procedure to reduce the duration of exposure to an infectious agent or chemical, to prevent dissemination of an infectious agent, and to reduce overall infection rates in a health care facility. Hand contamination occurs during manipulation of specimens and during contact with work surfaces, telephones, and equipment. Laboratory personnel should wash their hands immediately after removing gloves, after obvious contamination, after completion of work, before leaving the laboratory, and before hand contact with nonintact skin, eyes, or mucous membranes (8).

Handwashing sinks should be located at each entry/exit door; if possible, the faucet should be operated by a knee/foot control. If these controls are not available, the faucet should be turned on and off using a paper towel. Handwashing should be performed using soap or an antiseptic compound, starting at the wrist area and extending down between the fingers and around and under the fingernails, and rinsed from the wrists downward. Recently, the Centers for Disease Control and Prevention recommended that in addition to traditional soap and water handwashing, health care personnel can use alcohol-based gels (8).

Personal Protective Equipment (OSHA 2001 Blood Borne)

Occupational Safety and Health Administration (OSHA) standards for exposure to blood-borne pathogens require the laboratory to provide personal protective equipment (PPE) for its employees. OSHA standards require that PPE be provided, used, and maintained for all hazards found in the workplace, including biological, environmental, and chemical hazards, radioactive compounds, and mechanical irritants capable of causing injury or illness through absorption, inhalation, or physical contact. Employees must be trained in the appropriate use of PPE for a specific task, the limitations of PPE, and procedures for maintaining, storing, and disposing of PPE.

Gloves

Gloves protect the wearer from exposure to potentially infectious material and other hazardous material and are available in material designed for specific tasks. Gloves must be provided by the employer and must be the proper size and appropriate material for the task. Due to latex hypersensitivity in some workers, only powder-free latex gloves should be used or gloves should be manufactured from nitrile, polyethylene, or other material.

Protective Clothing

Laboratory workers should wear long-sleeved coats or gowns that extend below the level of the workbench, and they should be worn fully closed. The material must be fluid resistant if there is any potential for splashing or spraying. Fluid-proof clothing (plastic or plastic lined) must be worn when there is the potential for soaking by infectious material. Laboratory workers should not wear laboratory clothing outside the laboratory. All protective clothing should be changed immediately when contaminated to prevent the potentially infectious material or chemical from contacting the skin. Coats and gowns should not be taken home for cleaning but should be laundered by the institution.

Face and Eye Protection

Face and eye protection should be used when splashes or sprays of infectious material or chemicals may occur. Equipment includes goggles, face shields, and splashguards. Face shields provide the best protection for the entire face and neck, although splashguards provide an alternative method. If only goggles are worn, the user should also wear an appropriate mask to prevent contamination of mucous membranes.

Handling Specimens (17–20)

1. Specimens with *gross internal contamination* must not be accepted. Place the specimen in a plastic bag to protect subsequent handlers. The test request slips should be bagged separately from the specimen (keep paperwork clean and uncontaminated). Wear gloves when handling the specimen, and wash your hands thoroughly when you finish handling the specimen. Notify the supervisor immediately about a contaminated specimen so that further corrective action may be taken.
2. Specimens to be centrifuged *must* be placed in a sealed container to prevent aerosols.
3. All specimens other than bacterial or fungal blood culture bottles to be set up for isolation of organisms must be processed in a BSC. *All* "open-system" work is to be done on an absorbent surface (i.e., a towel) in a BSC, using appropriate protective techniques. Towels should be changed daily. We recommend that the immediate working area of the towel be dampened with any recommended disinfectant to handle small spills (Fig. 11.3).

Processing Specimens (17, 18)

1. *All specimens are potentially pathogenic—always use careful techniques* (Tables 11.3 and 11.4).
2. All discard material used in processing of specimens is considered contaminated.
    A. Fluids used in the processing of specimens (buffers, etc.), as well as excess liquid specimens, should be poured into plastic screw-top autoclavable bottles and sterilized prior to disposal. A tongue depressor placed in the bottle can help to prevent splashing if liquid is poured down the slanting stick. These sticks can also be placed in disinfectant before being discarded. Disinfectants and incineration can also be used for decontamination of infectious materials.
    B. Reusable items (tissue grinders, bottles, etc.) are placed in an autoclave pan. Once the pan is full, place it (without a cover) in a large brown autoclave bag and staple shut. After being autoclaved, the reusable items can be cleaned.

Figure 11.3 Example of a commercially available disinfectant (Solucide) that can be used for countertops, small spills, and any hard surface that requires a disinfectant (www.med-chem.com). doi:10.1128/9781555819002.ch11.f3

C. Specimen containers and centrifuge tubes are disposable. These items should be placed in plastic autoclave bags and secured with masking tape for sterilization. Slides can also be placed in containers of liquid disinfectant prior to disposal. Remember, slides should be treated as sharps.

D. Pipettes can be placed in a covered discard pan containing 5% amphyl or placed in autoclavable containers for sterilization before discarding.

Spills

1. Disinfectants in use at present
   A. *Bleach solutions*. To be an effective disinfectant, working bleach solutions must contain >0.5% but <2% sodium hypochlorite. Hypochlorite concentration in household bleach varies by manufacturer. Many household bleach solutions contain 5.25% sodium hypochlorite, and a 1:10 dilution (5,000 ppm Cl) will produce a 0.53% hypochlorite solution. Use of bleach solutions with lower hypochlorite concentrations might not provide the proper level of disinfection. Each day, prepare a fresh 1:10 household bleach solution (3, 23).
   B. *Alcohols*. Alcohols generally act by coagulating proteins and as organic solvents. Rapid evaporation may lead to inadequate “killing” time. Although there are some conflicting reports, 70% solutions generally have the best microbicidal activity. Caution is advised because of flammability. DO NOT USE ALCOHOLS OR ALCOHOL-BASED SOLUTIONS ALONE TO DISINFECT SURFACE AREAS; EVAPORATION SUBSTANTIALLY DECREASES EFFICACY.
   C. Another option can be seen in Fig. 11.3.

Note Use disinfectants recommended for environmental surfaces, such as Environmental Protection Agency (EPA)-registered disinfectants effective against hepatitis B virus, human immunodeficiency virus (HIV), and other bloodborne pathogens, or use a 1:10 dilution of household bleach. EPA environmental disinfectant product registration information is available at http://www.epa.gov/oppad001/chemregindex.htm (accessed 9/11/2013).

2. Immediate actions
   A. Clear the area at once.
   B. Notify the supervisor and chief of microbiology.
   C. Assess the type of spill and degree of hazard involved.
   D. Determine the most effective and least hazardous approach to clean up and decontaminate.
3. “Dry spills” with no significant aerosol formation
   A. Flood the area with disinfectant solution.
   B. Soak up disinfectant and contaminated material with absorbent material (sand or paper towels), and dispose of it in a plastic biohazard bag or sealed container. Wear gloves for cleanup.
   C. If routine cleanup is not possible, the unit may have to be decontaminated with a sterilizing gas such as paraformaldehyde.
   D. Thoroughly wash the unit (if possible) after decontamination.
4. Liquid spills on the bench or floor
   A. If significant aerosols were formed, the area should be evacuated and not reentered for at least 1 h.
   B. Flood the area with disinfectant, and cover the spill with absorbent material (sand or paper towels). Wear gloves during cleanup.
   C. Dispose of absorbent and contaminated material in plastic bags or sealed containers, and autoclave.
   D. Thoroughly wash the area after cleanup.
5. Centrifuge spills
   A. Shut off the instrument, and evacuate the area at once.
   B. Do not reenter the area for at least 1 h until aerosols have settled.
   C. The person entering the area to clean up should wear protective clothing, gloves, and a mask.

TABLE 11.3 Potential routes of laboratory infection with human parasites

Organisms[a]	**Mode of transmission**	**Comments**
Intestinal protozoa		
Entamoeba histolytica[b] (C) *Entamoeba dispar* (C) *Entamoeba coli* (C) *Entamoeba hartmanni* (C) *Endolimax nana* (C) *Iodamoeba bütschlii* (C) *Blastocystis* spp.[b] (C) *Giardia lamblia*[b] (*G. duodenalis*, *G. intestinalis*) (C) *Dientamoeba fragilis*[b] (T, C[c])	Accidental ingestion of infective cysts, trophozoites, oocysts, or spores in food or water contaminated with fecal material. Also direct transfer of stool material via fomites (fecal/oral transmission). Cysts (10–100) are required to initiate an infection with intestinal protozoa. (These modes apply to all intestinal protozoa.)	Transmission becomes more likely when fresh stool specimens are being processed and examined; submission of fecal specimens in preservatives or fixatives would decrease the risks. (This applies to all intestinal protozoa.)
Cryptosporidium[b] (O)	Oocysts (as few as 30) are required to initiate an infection with *Cryptosporidium* spp.	Wearing gloves, using capped centrifuge tubes, and working in BSC would decrease the risk of acquiring *Cryptosporidium* infections; the use of potassium dichromate as collection fluid and use of sugar flotation on fresh stool are *not recommended* for *Cryptosporidium*.
Cyclospora cayetanensis[b] (O) *Cystoisospora* (*Isospora*) *belli*[b] (O)	Accidental ingestion of mature oocysts; infective doses not known, but between 10 and 100 suspected; *Cyclospora* oocysts require a few days to weeks to mature.	*Cyclospora* oocysts are sensitive to drying but highly resistant to disinfectants.
Microsporidia[b] (S)	The infective dose for microsporidial spores has not been documented; however, one assumes the number would be small.	It is well documented that microsporidial spores can be inhaled, thus initiating an infection.
Intestinal helminths		
Enterobius vermicularis (E)	Inhalation or ingestion of infective eggs	Very common in children, asymptomatic
Strongyloides stercoralis (L)	Skin penetration of infective larvae from stool material	Exposure would be very possible or likely when working with stool cultures or concentrates for recovery of helminth larvae.
Hymenolepis nana (E)	Ingestion of infective eggs (fecal/oral)	Ingestion of infective eggs can lead to the adult worm in humans.
Taenia solium (E)	Inhalation or ingestion of infective eggs could lead to cysticercosis	Exposure is very likely when working with gravid proglottids (ink injection for worm identification).
Blood and tissue protozoa		
Leishmania spp.	Direct contact or inoculation of infectious material from patient lesion; accidental inoculation of material from culture or animal inoculation studies	Culture forms or organisms from hamster would be infectious.
Trypanosoma spp.	Same as for *Leishmania* spp.	Cultures and special concentration techniques would represent possible means of exposure.
Plasmodium spp.	Accidental inoculation could transmit any of the five species; infectious dose would be approximately 10 infected RBCs.	Blood should always be handled carefully (avoid open cuts, etc.).
Toxoplasma gondii	Inhalation or ingestion of oocysts in cat feces (veterinary situation); accidental inoculation of tachyzoites from culture, tube of blood, animal isolation (mouse peritoneal cavity)	Although many people already have antibodies to *Toxoplasma*, indicating past exposure, there have been documented laboratory accidents where the individual became ill due to the large infecting dose.
Ectoparasites		
Pediculus spp.	Specimens submitted on hair could be easily transmitted in the laboratory.	Careful handling and fixation of the arthropods would prevent any potential problems with transmission.
Sarcoptes scabiei	Transmission via skin scraping, etc., is possible but not very likely.	Careful handling and preparation of specimens with KOH would tend to prevent any problems.
Dipterous fly larvae (myiasis)	Transmission could occur anywhere.	Protection from flies would solve the potential problem.

[a] C, cyst; T, trophozoite; O, oocyst; L, infective larvae; E, egg; S, spore.
[b] Potentially pathogenic intestinal protozoa.
[c] With the discovery of the *Dientamoeba fragilis* cyst, this stage may also represent a method of transmission.

TABLE 11.4 Risk assessment and exposure control plan for the parasitology laboratory[a]

	Exposure risk		Personal protective equipment[b]				Engineering controls[c]	
Parasitology task	**Blood and body fluid contact**	**Cultured biological agent exposure**	**Gloves**	**Lab coat or gown[d]**	**Face shield**	**Splash shield**	**BSC**	**Sharps containers readily accessible[e]**
Concentrate fecal specimens, prepare smears and wet mounts	Low	BSL-2[f]	R	Coat				Pipettes/sticks
Read fecal wet mounts	Low	BSL-2	R	Coat				Slides/coverslips
Prepare thick and thin blood films	High	BSL-2	R	Coat	A	A		Slides/coverslips
Perform blood concentrations	High	BSL-2	R	Coat	A			Pipettes
Read blood concentration wet mounts	Low	BSL-2	R	Coat				Slides/coverslips
Stain and read fecal or blood slides	Low	BSL-2		Coat				Slides/coverslips
Culture fecal protozoa	High	BSL-2	R	Coat				Pipettes
Culture blood parasites	High	BSL-2	R	Coat				Pipettes
Culture free-living amebae	High	BSL-2	R	Coat	A	A		Pipettes, slides/ coverslips
Examine arthropod specimens	Low	BSL-2	R	Coat				Slides/coverslips
Perform antigen detection/ PCR/DNA probes	High	BSL-2	R	Coat	A[g]	A[g]		Pipettes

[a] Adapted from reference 23, protocol 15.3.2 (Risk Assessment).

[b] Remove personal protective equipment when leaving the laboratory. R, required; A, one of the required alternatives.

[c] Recapping of needles is prohibited. Tubes should be carried in racks; alternatively, plastic tubes should be used. Wash hands when leaving the laboratory.

[d] Gowns with solid front and impervious to liquid. Many employers provide and launder gowns, thus eliminating the need for laboratory coats.

[e] Sharps include scalpel blades, pipettes, plastic loops, sticks, needles, syringes, slides, and coverslips.

[f] BSL-2, biosafety level 2.

[g] Vortexing or other splatter-generating steps require the use of a BSC or safety shield.

    D. If liquids are present, soak up in absorbent material and handle as above. If liquids are not present, clean the instrument and room thoroughly before resuming work.
    E. Wipe all surfaces with a recommended disinfectant.

6. Spills in incubators, autoclaves, or other closed areas
    A. Flood with disinfectants, and soak up liquids with absorbent material.
    B. Dispose of the material as specified above, if possible.
    C. If routine cleanup is not possible, the unit may have to be decontaminated with a sterilizing gas such as paraformaldehyde.
    D. Thoroughly wash the unit (if possible) after decontamination.

Disposal of Contaminated Materials (3, 24)

1. Autoclave screw-cap tubes before cleaning or discarding.
2. Discard specimens and cultures into containers with double plastic lining. Liners should be changed when the containers are about half full.
3. Place culture plates into "Biohazard" receptacles lined with autoclavable bags.
4. Materials or containers to be reused should be autoclaved before being cleaned. Place them in sealed and clearly labeled containers to minimize hazard to others before sterilization.
5. Any breakage of glass or leakage of contaminated materials should be reported to the supervisor or chief of microbiology at once for instructions on procedures for safe cleanup.

Standard Precautions

All laboratories should adhere to the concept of standard precautions, which state that all patients and all laboratory specimens are potentially infectious and should be handled accordingly (8, 24–26). This concept arose from the observation that infections are often unrecognized in patients.

"Standard precautions" replaces earlier terms such as Blood and Body Fluid Precautions, Universal Precautions, and Body Substance Precautions found in OSHA documents (8, 26). The OSHA documents place the emphasis on blood-borne pathogens such as HIV and hepatitis B and C viruses, whereas the concept of standard precautions recognizes that all infectious agents and all other potentially infectious material, except sweat, pose a risk to the health care worker (8).

OSHA identifies a number of practices that should be implemented to protect the worker from exposure to blood-borne pathogens, including an exposure control and risk assessment plan (7). Methods should be implemented to minimize exposure to infectious agents, to shield the laboratory worker from infectious material through a set of engineering and work practice controls, and to use personal protective equipment. In addition, the OSHA regulations require that employers provide hepatitis B vaccination and postexposure evaluation and follow-up; communicate the hazards to employees; and maintain appropriate records (8). Employees who decline immunization against hepatitis B virus are required to sign a hepatitis B vaccine declination form.

Note Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious.

1. All job positions with any exposure to blood and body fluids must be listed on an exposure form, which must match up with continuing education training records from the facility.
   - A. Training must be provided at no cost to the employee and during working hours.
   - B. Training shall be provided as follows:
     - a. At the time of initial assignment to job functions in which occupational exposure may occur
     - b. Within 90 days after the effective date of the standard
     - c. At least annually thereafter
2. A complete task assessment must be performed for employees who have any exposure; protective measures, including equipment, must be defined.
   - A. Engineering controls
   - B. Immunization programs
   - C. Work practices (hand washing and procedures for handling sharps)
   - D. Disposal and handling of infectious waste
   - E. Use of personal protective gloves, gowns, and goggles
   - F. Use of mouthpieces, resuscitation bags, or other ventilation devices
   - G. Use of disinfectants
   - H. Labeling and signs
   - I. Training and education programs
   - J. Postexposure follow-up

   Some of the specifics are as follows:

   - A. All procedures shall be performed to minimize splashing, spraying, spattering, and generation of droplets of these substances.
   - B. When there is occupational exposure, the employer shall provide, at no cost to the employee, PPE such as (but not limited to) gloves (including hypoallergenic gloves, glove liners, powderless gloves, or other alternatives), gowns, laboratory coats, face shields or masks, and eye protection. Other options might include mouthpieces, resuscitation bags, pocket masks, or other ventilation devices.
   - C. PPE is considered "appropriate" only if it does not permit blood or other potentially infectious materials to pass through or to reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes. The type and characteristics of outer garments depend on the tasks and degree of exposure anticipated.
   - D. All working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials. All spills shall be immediately contained and cleaned up by appropriate professional staff or others trained and equipped to work with potentially concentrated infectious materials.
   - E. Specimens of blood or other potentially infectious materials shall be placed in a container which prevents leakage during collection, handling, processing, storage, transport, or shipping (27–29).
   - F. Mouth pipetting or suctioning of blood or other potentially infectious material is prohibited.
   - G. Certified BSCs or other combinations of personal protection or physical containment devices shall be used for all activities that may pose a threat of exposure. BSCs shall be certified when installed, whenever they are moved, and at least annually.
3. Key questions that people ask concerning these rules are listed below (30).
   - A. How does OSHA define "occupational exposure"? "Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties"
   - B. What does "reasonably anticipated" mean?

"Actual contact would be expected during an autopsy or surgery. In these cases, blood or other potentially infectious materials come in direct contact with the employee's gloves or other protective clothing. In other cases, contact may not occur each time the task or procedure is performed, but when blood or other potentially infectious materials are an integral part of the activity, it is reasonable to anticipate that contact may result."

C. What should the exposure control plan contain?
"The Exposure Control Plan is a key provision because it requires the employer to identify the individuals who will receive the training, protective equipment, vaccination, and other provisions of the standard."

D. What is considered "appropriate" personal protective equipment?
"Gloves, gowns, lab coats, face shields or masks and eye protection, mouthpieces, resuscitation bags, pocket masks, or other ventilation devices. Such protection will be considered 'appropriate' only if it does not permit blood or other potentially infectious materials to pass through to or to reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used. The type and characteristics of outer garments will depend upon the tasks and degree of exposure anticipated."

E. Does this mean that protective clothing must be "impervious" or "fluid-proof"?
"Selection of personal protective equipment is performance-oriented. Selection of the type and characteristics of necessary personal protective equipment is based upon the exposure anticipated to be associated with the task. In those instances where such equipment was incapable of halting penetration of blood or other potentially infectious materials normally encountered during a procedure, then additional protection such as a plastic apron should be worn or used."

Note "Fluid-resistant" and "fluid-proof" no longer appear in the final rule.

F. How can the employer ensure that the employee uses appropriate PPE?
"It is not the intent that the employer 'watch over everyone's shoulder' for compliance, but there are reasonable policies and regulations that employees follow."

G. What does the standard say about accessibility, cleaning, repair, removal, and storage of PPE?
"The employer shall ensure that appropriate personal protective equipment in appropriate sizes is available and is accessible at the worksite; the employer shall clean, launder, and dispose of personal equipment; the employer shall repair or replace when necessary at no cost to the employee. If a garment is penetrated by potentially infectious materials, it shall be removed ASAP and replaced by clean garments. When these garments are removed, they must be placed in a designated area or container for storage, washing, decontamination or disposal."

H. What is the employer's responsibility if PPE or the hepatitis B vaccine is refused?
"The employer shall ensure that the employee uses appropriate personal protective equipment unless the employer shows that the employee temporarily and briefly declined to use personal protective equipment when, under rare and extraordinary circumstances, it was the employee's professional judgment that its use would have prevented the delivery of health care services or would have created an increased hazard to the safety of the worker or co-worker. When an employee makes this decision, the circumstance shall be investigated and documented in order to determine whether changes need to be made to prevent such reoccurrences."

If the employee declines to accept hepatitis B vaccination, a form indicating that fact must be signed by the employee and held in the file.

I. What training is required for employees?
"Training must be available at the worksite, at no cost to the employee, and during working hours. It shall be provided at the time of initial assignment to tasks where exposure may occur, within 90 days after the effective date of the Standard, and at least annually thereafter."

J. What type of training is required for personal protective equipment?
"An explanation of the use and limitations of methods that will prevent exposure is required, including engineering controls (biosafety cabinets), work practices, and personal protective equipment. Information on types, proper use, location, removal, handling, decontamination, and disposal of personal protective equipment must be presented, in addition to an explanation of the basis for selection of such controls."

Hepatitis Exposure Protocol (4)

Laboratory personnel have a high risk of contracting hepatitis for the following reasons:

1. Frequent close personal contact with patients with hepatitis
2. Direct and frequent contact with biological specimens containing the virus
3. Frequent opportunity for accidental puncture wound with contaminated needles and sharp objects
4. Carelessness in handling specimens
5. Inadequate or unsafe disposal of contaminated needles, specimens, or other objects
6. Carelessness in proper and frequent hand washing technique

The prevention of hepatitis is the primary reason for the emphasis on infection control precautionary measures found in any safety manual (e.g., no smoking, eating, drinking, or mouth pipetting, and procedures for safe specimen handling). These same procedures also apply to HIV.

All known hepatitis specimens must be so labeled; however, to minimize exposure to the virus, *every specimen should be handled as though it could transmit hepatitis*. Studies have shown that hepatitis may be transmitted to laboratory personnel in several ways:

1. Puncture or other wounds (HIV)
2. Abrasions of the skin (HIV)
3. Aerosols (inhaled into the respiratory tract)
4. By mouth (oral route)
5. Direct contact with the patient
6. Splashing material into the eyes

The following protocol should be followed in the event of exposure:

1. Immediately wash the exposed area with soap and water.
2. Report the incident to the supervisor and laboratory personnel office.
3. Consult with a physician in Employee Health Facility concerning possible recommendations (e.g., administration of immune serum globulin).

Dangerous Properties of Industrial Materials

OSHA requires each laboratory to develop a comprehensive, written chemical hygiene plan (CHP). Every hazardous chemical in the laboratory, regardless of the type of risk, volume, or concentration, must be included in the CHP. The plan should include storage requirements, handling procedures, location of OSHA-approved material safety data sheets, and the medical procedures that are to be followed if exposure occurs. The CHP must specify the clinical signs and symptoms of the environmental conditions (such as a spill) that would give the employer reason to believe that exposure had occurred. When such conditions exist, the CHP should indicate the appropriate medical attention required.

Ethyl ether (also known as ether, anesthesia ether, diethyl ether, ethyl oxide, sulfuric ether, and ethoxyethane) is so volatile that dangerous concentrations are readily built up in the laboratory atmosphere. It is highly flammable and has a tendency to form explosive peroxides. Its low flash point, low ignition temperature, wide explosive range, high volatility, and very heavy vapor (which tends to "pocket") combine to make ethyl ether an extremely serious fire hazard. The spontaneous formation of explosive peroxides presents severe risks to the user who does not apply precautionary practices. Therefore, it is mandatory to follow these safety procedures (31, 32).

1. *Date* cans of ether immediately upon receipt.
2. *Do not attempt to open* a can that is 1 year old (after time of receipt); peroxides may form in sealed cans and explode when the cans are opened. Order appropriate quantities to avoid expensive waste.
3. *Date* the can of ether when opened; do not use after 1 month. Order cans of appropriate size to avoid waste.
4. *Store opened (corked) cans* of ether close to the floor, in a cool place, on a shelf, or in a cabinet that is *not* airtight. The heavy vapors can dissipate along the floor, with less opportunity for contact with sources of ignitions such as an open flame and electric sparks.
5. *Never store* opened cans of ether in a refrigerator, since ether still vaporizes at refrigerator temperatures. If peroxides have formed from ether, merely opening the refrigerator door may trigger an explosion. The heavy vapors that collect in an airtight refrigerator may ignite on contact with the electrical refrigerator motor. This recommendation also applies to "shielded" refrigerators (those in which the electrical systems are protected).
6. Unopened cans may be stored with opened cans (near the floor on an open shelf or nonairtight cabinet), in a storage cabinet for flammable materials, or in a storage room for volatile substances.
7. Outdate and discard cans after storage for 1 month (if opened) or 1 year (if unopened).
8. Flush empty, or almost empty, ether cans with copious amounts of water before discarding. "Empty" cans have been known to explode! Do not flush more than the contents of a 0.25-lb (ca. 0.1-kg) can down the drain; consult the Research and Occupa-

tional Safety Office or the local fire department for proper disposal of large quantities. Empty, thoroughly rinsed cans may be discarded in the regular trash.

9. Do not attempt to open any containers of uncertain age or condition or those whose cap or stopper is tightly stuck. Peroxides have been known to form under the threads of caps! These containers should be discarded.
10. Unopened outdated cans must be delivered to Research and Occupational Safety for disposal (each institution may have a comparable office or safety office).
11. *Certain chemicals can be replaced by others that are safer* (33, 34). Ethyl acetate, with a higher flash point (4.0°C), has replaced ether (flash point of −45°C) in the formalin-ether concentration technique. Hemo-De (Medical Industries, Los Angeles, CA, or Fisher Scientific, Los Angeles, CA) can also replace ethyl acetate in the concentration procedure. Hemo-De has a flash point of 57.8°C and is generally regarded as safe by the U.S. Food and Drug Administration. Xylene, as used in the trichrome or iron hematoxylin staining of polyvinyl alcohol (PVA)-fixed fecal smears, poses potential toxic and fire hazards. Again, Hemo-De has successfully replaced xylene in both the carbol-xylene and xylene steps of the trichrome procedure (33). Other substitutes are available as well (e.g., Hemo-Sol [Fisher Scientific]). Check with your local pathology departments or reagent suppliers for other alternatives.
12. Mercuric chloride, used in PVA and in Schaudinn's fixative, presents both a toxic hazard and a disposal problem. Copper sulfate has been suggested as a substitute for mercuric chloride (35, 36); however, protozoan morphology is not as clear and precise when this formula is used. The use of zinc rather than copper gives fairly good morphology when the trichrome stain is used (35).

Note Information on other substances is provided in Table 11.5.

Current OSHA Regulations for the Use of Formaldehyde

Formaldehyde has been in use for over a century as a disinfectant and preservative; it is also found in a number of industrial products. There is disagreement about the carcinogenic potential of lower levels of exposure, and epidemiologic studies of the effects of formaldehyde exposure among humans have given inconsistent results. Studies of industry workers with known exposure to formaldehyde report little evidence of increased cancer risk (37). It also appears that persons with asthma respond no differently from healthy individuals following exposure to concentrations of formaldehyde up to 3.0 ppm (38).

OSHA requires all workers to be protected from dangerous levels of vapors and dust. Formaldehyde vapor is the most likely air contaminant to exceed the regulatory threshold in the clinical laboratory (39, 40). Current OSHA regulations require vapor levels not to exceed 0.75 ppm (measured as a time-weighted average [TWA]) and 2.0 ppm (measured as a 15-min short-term exposure). *OSHA requires monitoring for formaldehyde vapor wherever formaldehyde is used in the workplace. The laboratory must have evidence at the time of inspection that formaldehyde vapor levels have been measured, and both 8-h and 15-min exposure must have been determined.*

If each measurement is below the permissible exposure limit and the 8-h measurement is below 0.5 ppm, no further monitoring is required as long as laboratory procedures remain constant. If the 0.5-ppm 8-h TWA or the 2.0-ppm 15-min level is exceeded, monitoring must be repeated semiannually. If either the 0.75-ppm 8-h TWA or the 2.0-ppm 15-min level is exceeded (unlikely in a clinical laboratory setting), employees must be required to wear respirators. Accidental skin contact with aqueous formaldehyde must be prevented by the use of proper clothing and equipment (gloves and laboratory coats).

The amendments of 1992 add medical removal protection provisions to supplement the existing medical surveillance requirements for employees suffering significant eye, nose, or throat irritation and for those experiencing dermal irritation or sensitization from occupational exposure to formaldehyde. In addition, these amendments establish specific hazard-labeling requirements for all forms of formaldehyde, including mixtures and solutions composed of at least 0.1% formaldehyde in excess of 0.1 ppm. Additional hazard labeling, including a warning label that formaldehyde presents a potential cancer hazard, is required where formaldehyde levels, under reasonably foreseeable conditions of use, may potentially exceed 0.5 ppm. The final amendments also provide for annual training of all employees exposed to formaldehyde at levels of 0.1 ppm or higher (41).

Note The use of monitoring badges may not be a sensitive enough method to correctly measure the 15-min exposure level. Contact the Occupational Health and Safety Office within your institution for monitoring options. Usually, the accepted method involves monitoring airflow in the specific area(s) within the laboratory where formaldehyde vapors are found.

TABLE 11.5 Potential hazards of industrial substances[a]

Compound	Synonym	Hazard
Acids and bases		Extremely irritating and corrosive to the skin and mucous membranes. Can quickly cause burns and deep ulceration with ultimate scarring. Prolonged contact with dilute solutions has a destructive effect on tissue.
Ethyl acetate	Acetic ether	Absorbed through intact skin. Irritating to mucous surfaces, particularly the gums, eyes, and respiratory passages; is also mildly narcotic. Repeated or prolonged exposures cause conjunctival irritation and corneal clouding.
Formalin	Formaldehyde solutions	Severe local irritant. If swallowed, causes violent vomiting and diarrhea.
Mercury compounds		Local irritants and systemic poisons. Absorbed through intact skin. Strong allergens.
Methyl alcohol	Methanol	Absorbed through intact skin. Acutely, there is little or no danger, but with chronic use a toxic effect may develop because, once absorbed, it is eliminated very slowly, so that a cumulative effect can result with repeated low dosage.
Phenol	Carbolic acid	After skin contact, the area becomes white, wrinkled, and softened, and there is usually no immediate complaint of pain; later, intense burning is felt. Absorption is through intact skin and can be quite rapid. Upon spillage, death can occur within 30 min. In acute phenol poisoning, the main effect is on the central nervous system.
Potassium dichromate	Potassium bichromate	*Carcinogen.* Has a very corrosive action on the skin and mucous membranes. The ulcers produced are frequently painless and are slow to heal. Associated with cancer of the lungs.
o-Tolidine	3,3-Dimethylbenzidine	*Carcinogen. One of the many aromatic amines that are recognized as carcinogens to the human bladder, ureter, and renal pelvis and suspected carcinogen to the intestines, lungs, liver, and prostate.*
o-Toluidine	*o*-Methylaniline	*Carcinogen.* While it can be absorbed through intact skin, the main portal of entry is by inhalation. Can produce severe systemic disturbances.
Xylene	1-Dimethyl benzene	Acute toxicity may be greater than that of benzene or toluene. Absorption takes place chiefly through the lungs. Skin irritation may be serious. Extended exposure can cause gastrointestinal neurologic and tissue damage.

[a] Data from Sax NI, *Dangerous Properties of Industrial Materials*, 4th ed., Litton Education Publications, Van Nostrand Reinhold, New York, NY, 1975.

Latex Allergy

The incidence and evidence of clinical latex sensitivity appear to be increasing since its first description in 1979. Since the introduction of standard precautions, the use of latex gloves by health care workers has increased dramatically (7). Those at risk appear to be health care workers and employees working in latex industries, patients with atopic diathesis, and patients who underwent repeated surgical procedures during childhood (42). The allergic response occurs to one or more natural rubber latex proteins, resulting in contact urticaria, angioedema, allergic rhinitis, asthma, or anaphylaxis. Early recognition of sensitization to latex is crucial to prevent the occurrence of life-threatening reactions in sensitized health care providers or patients (43). Latex allergy is now an important medical, occupational, medicolegal, and financial problem, and it is essential that policies be developed to reduce the problem (44–46). There also seems to be a relationship between allergies to natural latex rubber and allergies to avocados, chestnuts, and bananas. A high incidence of latex sensitivity has also been found in people with spina bifida (47).

The use of cornstarch powder in gloves can sensitize healthy people and exacerbate the symptoms of allergic patients. The powder spreads the latex allergens into the environment (42). Accompanying this increase in latex allergy is evidence of positive circulating specific immunoglobulin E antibodies to latex (48). Unfortunately, the primary treatment for latex allergy is avoidance (49).

Quality Systems

The area of quality systems forms an integral part of any clinical laboratory operation. In many cases of clinical laboratory testing, various programs and guidelines for quality assurance procedures (including QC) have been thoroughly outlined. However, specific quality assurance recommendations within the parasitology section of microbiology have not been well defined in the past, and the information in this chapter should help clarify these recommendations. Although the diagnostic procedures in this area seldom yield numerical data, there are still a number of QC measures that can help to ensure accurate results.

Extent of Services

The range of services provided will vary from one laboratory to another. To comply with the Health Care Financing Administration (HCFA) guidelines, only two extent classifications (rather than the previous four) are recognized in parasitology by the College of American Pathologists (50).

1. *Extent 2.* "Limited parasitology performed. The laboratory is able to recognize the presence of parasites, including protozoa in clinical specimens, but may need to refer them for definitive identification. When permanently stained smears for intestinal protozoa are indicated, polyvinyl alcohol fixative (PVA) or other appropriate fixative is available, and such material is placed in a fixative and then referred to a reference laboratory for staining identification."
2. *Extent 3.* "Definitive identification of parasites present to the extent required to establish a correct clinical diagnosis and to aid in selection of safe and effective therapy. Laboratories are expected to have a high degree of expertise and should be able to differentiate *Plasmodium falciparum* from other species of *Plasmodium.*"

Note There is no longer a College of American Pathologists listing for extent 1 (no parasitologic procedures are performed and all specimens are submitted to a reference laboratory). However, in this situation, the laboratory would not be required to subscribe to outside proficiency testing specimens in this discipline.

Proficiency Testing

Based on the Extent of Services mentioned above and the requirements in the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88), all (with rare exceptions) clinical laboratories (including physician office laboratories performing other than waived tests) must participate in an approved program of interlaboratory comparison testing that is consistent with the level and complexity of the work performed. The comprehensive microbiology and/or parasitology survey programs are recommended. The state Department of Health must be consulted to confirm which proficiency testing services fulfill the requirements related to a laboratory's particular accreditation, particularly if the laboratory accepts interstate specimens in parasitology.

There must be evidence of active review of the survey results by the laboratory director or the designated supervisor. There also must be written evidence of evaluation of the results and, if indicated, corrective action when unacceptable results have been reported. Corrective action statements should review the correct answer and specific steps that will be taken to ensure that the same, or similar, error will not occur in the future. On the basis of the regulations within CLIA '88, HCFA or one of the HCFA-approved agencies (may include some state agencies) will be involved in the process of monitoring laboratory performance and coordinating any possible sanctions that might be involved as a result of unacceptable performance in proficiency testing programs.

Copies of the 28 February 1992 *Federal Register* containing the final CLIA regulations can be obtained by writing to Government Printing Office, Attn: New Order, P.O. Box 371954, Pittsburgh, PA 15250-7954. Specify the date of the issue requested (28 February 1992) and stock number (069-001-00042-4). A $3.50 check or money order payable to "Superintendent of Documents" or a Visa or MasterCard number and expiration date should be enclosed. Orders may be made by telephone [(202) 783-3238] or fax [(202) 512-2250].

It is recommended that all of the proficiency testing specimens be saved for future review, particularly when students are being trained or technologists, pathologists, and residents are receiving review training. It is just as important to maintain negative specimens as to maintain positive ones, since many errors are made in which artifacts are identified as parasites. Specimens should be periodically checked to make sure that the volume of fixative is sufficient to prevent the specimens from drying out. These specimens should be cataloged for easy reference.

Specific recommendations for the handling and examination of proficiency testing specimens can be found in Table 11.6. These guidelines should assist the participant in the proper approach to these specimens.

CLIA '88 mandated universal regulation for all clinical laboratory testing sites in the United States, including previously unregulated sites in physician offices. Quality testing was to be achieved through a combination of total quality management and mandated minimum quality practices. Through both internal and external proficiency testing, performance standards were also mandated. After the implementation of CLIA '88, the percentage of laboratories passing proficiency testing requirements has improved and almost all laboratories are currently using quality practices for a more comprehensive quality system approach (51, 52).

Survey results from the Laboratory Proficiency Testing Program, Toronto, Ontario, Canada, over the course of the program, which began in 1977, indicate a marked improvement in laboratory performance. They send four samples in each of four surveys per year consisting of specimens to be examined for gastrointestinal parasites. Improvement is thought to result from a combination of voluntary withdrawal by laboratories with poor performance and improved performance by the other laboratories. Extensive educational initiatives have also played a significant role in improved performance (53).

TABLE 11.6 Recommendations for the handling and examination of proficiency-testing specimens

Specimen	Method	Comments
Formalin	Direct examination	Although some directions recommend sampling the vial after mixing, if organism numbers are small, they may be missed. Another option is to allow the vial to stand on the counter for a minimum of 5 to 10 min, then take one small drop from the bottom of the vial and prepare the coverslip (usually 22 by 22 mm). Make sure you do not introduce bubbles via the pipette; if this occurs, allow the vial to stand for another 5 to 10 min before sampling the bottom layer. If iodine is used, make sure the overall color is not too dark; helminth eggs may appear to be debris. The color should resemble strong tea. There is normally not sufficient material to perform a concentration procedure; this is not recommended. The entire coverslip preparation should be examined with the 10× low-power objective. At least 1/4 to 1/3 of the area should be examined with the 40× high dry objective. The use of immersion oil is not recommended for these preparations.
PVA fecal smear	Permanent stain (trichrome or iron hematoxylin)	Although many laboratories have switched to nonmercury fixatives, it is important to remember that some proficiency-testing fecal specimens for permanent staining have been preserved in mercury-based fixatives. This means that *you must include the iodine and subsequent alcohol rinse steps in your staining method to remove the mercury from the smear.* If this is not done, the overall result may be very poor. Both trichrome and iron hematoxylin staining methods can be used for these smears. At least 300 fields should be examined with the 100× oil immersion objective. These stained smears can be examined with a 50× or 60× oil immersion lens, but final organism identification often requires the 100× oil immersion objective. Consult your proficiency testing agency for mercury or nonmercury use. If nonmercury fixatives are used, then the iodine/subsequent rinse step can be omitted from the staining procedure; the first step would be a simple 70% alcohol rinse and then into the stain. However, the first step could also be directly into the stain with the dry slide.
Formalin or fecal smear (non-PVA)	Modified acid-fast stain	Special staining is required if the patient history is suggestive of possible cryptosporidiosis. Unless there is some indication (history of immunosuppression), it is unlikely that *Cryptosporidium* is a possibility. If you perform a modified acid-fast stain, either the hot or cold method is acceptable. Make sure that the destaining step is not too harsh; do not use acid-alcohol, commonly used in the routine Ziehl-Neelsen acid-fast stain. Generally, a mild destain is recommended (1% sulfuric acid). Although the organisms can be seen with the low-power (10×) objective, it is recommended that the high dry (40×) objective be used. The 50× oil immersion objective is also an accepted approach to screening the smear.
Thin blood film	These films arrive already stained.	The *entire blood film* (including the area where the drop was placed on the slide) should be carefully examined with the 10× low-power objective. Often, when these films are prepared, any microfilariae present are at the edges of the blood film or in the thick portion of the film. Without this type of examination, these microfilariae may be missed. With the 100× oil immersion lens, a minimum of 300 oil immersion fields should be examined. These stained blood films can be screened with a 50× or 60× oil immersion lens, but final organism identification often requires the 100× oil immersion objective. If young malarial rings are present, they could be missed if only the lower-power oil immersion lens is used.
Thick blood film	These films arrive already stained.	The entire thick blood film should be examined with the 10× low-power objective. When these films are prepared, any microfilariae present may be at the edges of the blood or in any portion of the thick film. Without this type of examination, these microfilariae may be missed. If microfilariae are seen, morphologic details can be seen using the high dry 40× objective. With the 100× oil immersion lens, a minimum of 300 oil immersion fields should be examined. These stained blood films can be screened with a 50× or 60× oil immersion lens, but final organism identification often requires the 100× oil immersion objective. If young malarial rings are present, they could be missed if only the lower-power oil immersion lens is used.
Antigen detection using fecal immunoassays	Fluorescent antibody assays, enzyme immunoassays, or the cartridge format tests are available.	Enzyme immunoassays (antigen detection, no centrifugation recommended) 1. Remember to thoroughly rinse the wells according to the instructions; do not eliminate any of the rinse steps. Make sure each well receives the total number of rinses required. 2. Make sure the stream of buffer goes directly into the wells. Use a wash bottle with a small opening, so you have to squeeze the bottle to get the fluid to squirt directly into the wells. 3. When the directions tell you to "slap" the tray down onto some paper towels to remove the last rinse fluid, make sure you slap it several times. Don't be too gentle; the cups will not fall out of the holder. If the cups become loose, push them down into the tray again. 4. Before adding the last reagents, the wells should be empty of rinse buffer (not dry, but empty of excess fluid).

(continued)

TABLE 11.6 Recommendations for the handling and examination of proficiency-testing specimens *(continued)*

Specimen	Method	Comments
		Fluorescence (visual identification of the organisms) 1. Since you will be looking for the actual organisms (cysts of *Giardia* and/or oocysts of *Cryptosporidium*), this test should be performed on centrifuged stool ($500 \times g$ for 10 min) to increase the sensitivity. 2. Remember to thin out the smear; it is important to make sure that the slides are thoroughly dry before adding reagents. The slides can be placed in a 35°C incubator for about 30 min to 1 h to make sure that they are dry before being processed. If the material on the wells is too thick, it may not dry thoroughly and may fall off the glass. It is better to let them dry longer rather than for too short a time. A heat block is *not* recommended for this purpose. 3. *Gently* rinse the reagents from the wells; do not squirt directly into the wells, but allow the rinse fluid to flow over the wells. 4. Remember that not all clinical specimens provide the 3+ to 4+ fluorescence that we often see in the positive control. Also, from time to time, you may see fluorescing bacteria and/or some yeast in certain patient specimens. This is not that common, but the shapes can be distinguished from *Giardia* cysts and/or *Cryptosporidium* oocysts. The intensity of the fluorescence may vary, depending on the filters. If your filters demonstrate both the yellow-green fluorescence and the red-orange counterstain, the organisms may not appear quite as bright as when the yellow-green filter only is used. Both approaches are acceptable and may reflect personal laboratory preferences. 5. Make sure to examine the edges of the wells. Sometimes in a light infection, the edges contain organisms while the organisms in the middle of the well may be a bit more difficult to detect (thick area). 6. Since the *Giardia* cysts and trophozoites share some epitopes, you occasionally may see pale fluorescence for a trophozoite; this can be interpreted as a positive test. Fluorescence is generally around the 1+ level.
		Lateral-flow cartridges (antigen detection, no centrifugation recommended) 1. If the stool is too thick, the addition of reagents will not thin it out enough. If the specimen poured into the well remains too thick, the fluid will not flow up the membrane. If your specimens arrive in fixative and there is no fluid at the top of the vial overlaying the stool, this means that the vial may have been overfilled with stool. These specimens will have to be diluted with the appropriate diluent before being tested. 2. It is always important to see the control line indicated as positive all the way across the membrane, not just at the edges. 3. A positive test result is generally lighter than the control line; this is normal. 4. At the cutoff time to read the result, any color at all visible in the test area should be interpreted as a positive. 5. Do not read/interpret the results after the time indicated in the directions; you may get a false-positive result.
Photomicrograph	Project to view	These 2 × 2 photomicrographs are generally accompanied by some type of history or measurements of the object. It is important to actually project the image. Observing the slide through a small slide holder may not reveal sufficient detail for the accurate identification of the specific organism shown in the slide.
Computerized digital image	Follow examination directions	These images generally have specific fields (10 to 12) marked for the participant to determine the identification of the organism within the box. Make sure you take advantage of the viewing aids (size, contrast, brightness) in the program. Generally, each slide contains a single organism for identification; however, in future challenges there may be dual infections present for identification.
All specimens	Data entry	It is important to remember to very carefully check your responses and entry of choices onto the appropriate forms. When being graded, your responses will be evaluated on data submitted. If the incorrect answer is marked, you may be penalized, depending on consensus and a number of other parameters. Prior to submitting data, familiarize yourself with the paperwork and information requested. It is important for the laboratory to establish some type of tracking system for these specimens. You should have a log book or something similar where the arrival date and due date for each proficiency-testing module can be clearly entered. One person should be responsible for communicating with the agency in the event of breakage, failure of the shipment to arrive, etc. Each agency will give you at the beginning of the year an anticipated schedule of dates for shipment of each set of samples for testing.

In-House Quality Control

Supervision

The QC program should be under surveillance by the chief technologist or section supervisor and should be reviewed at least once each month by the laboratory director, QC supervisor, or other supervisor designate. There should be written evidence of active review of all records (controls for routine procedures, instrument function tests, and equipment checks [temperature, humidity, systems, maintenance]). There should also be written evidence of corrective action when controls do not fall within acceptable limits. A written checking system should be in operation to help detect clerical errors, analytical errors, and/or unusual laboratory results. This system should also provide for error correction within acceptable time frames. With the emphasis on outcome-oriented measures, it is very important to have the capability to track testing through the preanalytical, analytical, and postanalytical phases (specimen integrity, processing, testing, reporting, and consultation), with primary emphasis being placed on accurate, reliable, and timely diagnostic testing for good patient outcome. This approach to laboratory inspections is emphasized by HCFA or other HCFA-designated agencies for outside inspections and should also be emphasized in all in-house quality assurance programs.

Procedure Manuals

Although card files and wall charts are acceptable for quick review, a well-written and complete procedure manual is very important for the routine operation of the laboratory and mandatory for accreditation requirements (Tables 11.7 and 11.8). Instructions contained in manufacturers' package inserts are helpful but do not fulfill the requirements for a procedure manual. It is highly recommended that the National Committee for Clinical Laboratory Standards (NCCLS; now the Clinical and Laboratory Standards Institute [CLSI]) format be followed for protocol preparation (54). This publication covers the design, preparation, maintenance, and use of technical procedure manuals for the clinical laboratory. The procedure manual should contain for each test: (i) the principle of the test; (ii) acceptable specimens (including rejection criteria) and instructions for proper specimen collection and processing; (iii) preparation of reagents and solutions; (iv) all supplies and equipment necessary to perform the test; (v) QC procedures, results, and interpretation (including corrective action if QC is out of control); (vi) the test method; (vii) possible results; (viii) the correct way to report results; (ix) procedure notes (tips); (x) limitations of the procedure; (xi) additional tables and charts; and (xii) references. Complete parasitology protocols (written according to the NCCLS guidelines) can also be found in the *Clinical Microbiology Procedures Handbook* (1).

Protocols are divided into three sections: *preanalytic*, *analytic*, and *postanalytic*. The *preanalytic phase* includes steps taken before the test is actually performed (pre-examination phase). Processes begin with the test request and its format, patient preparation, specimen collection, specimen transportation, specimen receipt in the laboratory, and specimen processing. The *analytic phase* contains all activities related to the performance of laboratory testing or examination of specimens. The *postanalytic phase* contains the steps that occur after a laboratory test has been performed. Processes include data review, report formatting,

TABLE 11.7 Requirements for technical procedure manuals[a]

Requirement
General
Manual must be written; textbook not acceptable
Complete and current; contains only those tests performed in the section
Reviewed and approved annually
Changes dated and approved
Available at the bench
Specimen
Patient preparation
Specimen collection
Specimen handling
Specimen storage
Criteria for unacceptable specimen; notification process
Quality control
Preparation of controls
Mean value and upper/lower limits
Lot number and date of preparation
Remedial action to be taken with unacceptable results
Procedure
Preanalytical considerations
Principle of test
Specimen (see above)
Materials (reagents and supplies)
Preparation and storage of reagents and standards
Analytical considerations
Quality control (see above)
Methodology
Calibration and linearity
Derivation of results
Postanalytical considerations
Reporting results
Alternate procedure for automated tests
Normal ranges and results
Criteria for handling abnormal and alert values
Flow sheets, keys, tables
Procedure notes
Limitations of the procedure
References and supplemental reading

[a] Regulatory agencies or relevant laws: Clinical Laboratory Improvement Act (CLIA/Medicare) 1967; College of American Pathologists (CAP), 1978; American Association of Blood Banks (AABB) 1981; The Joint Commission (JCAHO) 1980; CLIA 1988.

TABLE 11.8 Quality assessment of postanalytic systems

Quality assessment of postanalytic systems should include review or evaluation of the following areas[a]:

- Effectiveness of corrective actions
- Procedures and policies to prevent recurrences
- Accuracy and completeness of test results and reports
- Disposition of unacceptable specimens
- Turnaround times
- Referral specimens and their reports
- Corrected reports
- Procedures for notification of test results, including STAT tests
- For "Analyte Specific Reagents," test reports should include information necessary for proper interpretation of results, including disclaimers as needed
- Ensure confidentiality of patient information

[a]Adapted from reference 4.

result interpretation and presentation, permission for release, result transmission, and sample storage.

Specimen Handling and Record Storage

All specimens must be recorded in an accession book, log book, worksheet, computer, or other comparable record. If specimens are not examined as fresh material, they should be preserved in appropriate fixatives for later examination. On the basis of current guidelines, it is recommended that specimen and test result records be kept for at least 2 years (longer for pathology records and those from the blood bank). Also, remember that when your laboratory is inspected, you must be able to retrieve those records within no more than a few hours; this is particularly important if the inspector is actually checking this parameter.

Test Request Requisitions

All requisitions should be designed to allow sufficient information for patient and physician identification and any clinically relevant information. Specific items should include (i) patient name, (ii) patient identification number, (iii) name of ordering physician, (iv) source of specimen, (v) specific procedure(s) ordered, (vi) time of specimen collection, and (vii) time of receipt or initial processing by the laboratory. Other helpful information, particularly in the parasitology division, is (i) suspected diagnosis, (ii) travel history, and (iii) recent medication. Since this information is often not on the original requisition, the physician may have to be contacted for clarification.

Procedure QC and Documentation

QC measures for all procedures are included in the individual protocols within this section of the book. Not only must the QC checks be performed as indicated, but also complete documentation should be generated, including specific plans for out-of-control results and problem resolution. Result expectations and normal results or values must be specifically defined and posted on any QC check sheets used in the laboratory. It is not sufficient to merely check a column that QC is within acceptable limits; you must also have on these sheets a definition of what normal or "acceptable limits" means. Dates and space for the initials or name of the person performing the QC checks must also be included. Specific procedure recommendations include the following (1, 6, 55, 56)

1. The examination of formed stools should include a concentration procedure.
2. A permanent stained smear is recommended for *every stool specimen* submitted for testing (liquid, soft, or formed).
3. A direct wet mount should be performed for fresh stool specimens (liquid or mushy—not semiformed or formed), in addition to the concentration and permanent stained smear. Since the direct wet mount is designed to allow the detection of organism motility, it is not necessary to perform the direct wet mount on preserved specimens or semiformed/formed stools. Consequently, if the stool specimens are received in preservative, proceed directly to the concentration and permanent stained smear.
4. Both thick and thin blood films are prepared for examination for malarial parasites.
5. Stained smears are washed with buffered distilled water (pH 6.8 to 7.2); any of the blood stains can be used (Giemsa, Wright/Giemsa, Field's, rapid stains).
6. Adequate numbers of oil immersion fields should be examined (at least 300) on both stained blood films and permanent stained fecal smears.

Test Result Reports

With few exceptions, the following methods of reporting are acceptable.

1. All parasites should be reported, regardless of whether they are considered nonpathogenic or pathogenic. The presence of any parasites within the intestine generally confirms that the patient has acquired the organism through fecal-oral contamination.
2. Generally, protozoa and helminths are not quantitated on the laboratory slip. However, the specific stage (i.e., trophozoites, cysts, oocysts, spores, eggs, or larvae) is indicated.
3. Exceptions to quantitation would be *Blastocystis* spp. (there may be some association between numbers and symptoms) and *Trichuris trichiura* (light infections may not be treated).
4. The complete genus and species name of the organism identified should be reported.

TABLE 11.9 Quantitation of organisms[a]

	Quantitation	
Category	**Protozoa**	**Helminths**
Rare	2–5 per cslp[b]	2–5 per cslp[c]
Few	1 per 5–10 hpf	1 per 5–10 lpf
Moderate	1–2 per hpf to 1 per 2–3 hpf	1–2 per lpf
Many	Several per hpf	Several per lpf

[a] This quantitation (based on a 22- by 22-mm coverslip) is used by the Centers for Disease Control and Prevention, Proficiency Testing Program, Microbiology–Parasitology, 1985. Abbreviations: cslp, 22- by 22-mm coverslip; hpf, high-power field (magnification, ×400); lpf, low-power field (magnification, ×100).

[b] Total coverslip with high dry power (magnification, ×400).

[c] Total coverslip with low power (magnification, ×100).

5. Charcot-Leyden crystals should be reported and quantitated.
6. If the specimen is fresh or freshly preserved, budding yeast cells should be mentioned and quantitated (consultation with the physician recommended).
7. All quantitation should be consistent (Table 11.9).
8. Helminth eggs and larvae should be reported.
9. Special circumstances (additional specimens required to rule out this infection, etc.) require additional comments on the laboratory report.
10. Remember, the name of the laboratory actually performing the procedures must be indicated on the final report or elsewhere.

Reagents

All reagents should be properly labeled with (i) content, (ii) concentration, (iii) date prepared or received, (iv) lot number, (v) date placed in service, and (vi) expiration date. If reagents are prepared in-house, you should also have recorded (i) the name of person preparing the reagent and (ii) the date and results of the QC check.

The specific gravity of certain solutions (e.g., zinc sulfate) should be checked before use (this is particularly important if the solution is used infrequently). The hydrometer should have a scale large enough to differentiate between specific gravities of 1.18 for use with fresh specimens and 1.20 for use with formalinized specimens. This type of solution should be kept in a tightly stoppered bottle.

Stains should be routinely checked with control specimens for correct staining properties before use. Certainly, new lot numbers or new batches of stain should be checked. Also, if reagent use is infrequent, the stains should be checked before use. The same rule generally applies to fixatives.

With these basic recommendations in mind, the following specific QC methods can be used. While not every reagent used in diagnostic parasitology work is reviewed, these methods can be adapted to other reagents.

The examination of fecal material for ova and parasites may reveal, in addition to parasitic organisms, cells of human origin (blood cells). Most of these cells are not usually found in the stool; however, they may be found in the presence of parasites and/or other disease-producing organisms. These cells may occur in the stool without the presence of a specific etiologic agent (allergic conditions, ulcerative colitis, etc.). The buffy coat cells (white blood cells [WBCs]) can also be used as a QC check for various fixatives. The WBCs normally found in the buffy coat portion of whole blood include polymorphonuclear neutrophils, eosinophils, lymphocytes, and monocytes (macrophages). When blood is visible in the stool (indicating the presence of fresh blood), the WBC morphology is very similar to that seen on the stained thin blood film used for a differential. However, if the blood has been in the gastrointestinal tract for some time, the cellular morphology changes slightly.

QC for Schaudinn's Fixative

1. Collect a fresh tube of blood (lavender top, EDTA anticoagulant), or get a tube from the hematology section (high WBC count, if possible).
2. Centrifuge the blood, and remove the buffy coat (layer containing WBCs).
3. Add the buffy coat to approximately 2 to 4 g of fresh, soft stool, and mix thoroughly *but gently*.
4. This stool-WBC mixture can be smeared onto several slides and immediately immersed in Schaudinn's fixative.
5. After staining, if the WBCs appear well fixed and typical morphology is visible, one can assume that any intestinal protozoa placed in the same lot number of Schaudinn's fixative would also be well fixed, provided that the fecal sample was fresh and fixed within recommended time limits.

QC for Fixative containing PVA

1. Mix approximately 2 g of fresh, soft stool with 10 ml of fixative containing PVA (in solution, ready for use). The ratio of stool to fixative should be approximately 1/3 to 1/5; do NOT add too much stool to the vial.
2. To this fixative-PVA-stool mixture, add several drops of buffy coat cells (concentrated/collected as above) and mix gently.
3. After a 30-min fixation, pour a small amount of the fixative-stool-PVA-cell mixture onto a paper towel to absorb the excess PVA.
4. The material can then be smeared onto several slides, allowed to dry thoroughly (60 min at room temperature or 30 min at 35°C), and stained. After staining, if the WBCs appear well fixed and typical

morphology is visible, one can assume that any intestinal protozoa placed in the same lot number of PVA fixative would also be well fixed provided that the fecal sample was fresh and fixed within recommended time limits.

Note *Entamoeba coli* mature cysts are very difficult to fix properly and may be difficult to identify on the stained smear. For this reason, it is possible to have fixatives that are adequately QC checked but do not always yield good morphology for this particular organism. Use of a longer fixation time (60 min) sometimes produces better morphology after staining.

Note The same approach can be used for other stool preservatives (sodium acetate-acetic acid-formalin [SAF], zinc- and copper-based fixatives, other single-vial collection systems, and the Universal Fixative/TOTAL-FIX).

QC for Stains (Trichrome, Iron Hematoxylin, etc.)

1. Slides may be prepared from a known positive fecal specimen preserved in PVA or SAF. These slides can then be stained and checked for typical organism morphology. Positive PVA-preserved samples can be purchased commercially; many slides can be prepared from one sample. QC slides (already prepared and ready for staining) are also available commercially.
2. Buffy coat cells (after appropriate fixation) may also be used to check the staining properties of each new lot number of stain.

Note Do not prepare too many slides in advance. If dry fecal smears containing PVA are held too long before staining, the organism or buffy coat cell morphology may be poor.

Instruments and Equipment

General requirements include the following: (i) all instruments and equipment should be on a routine preventive maintenance schedule, and records should be maintained in writing; (ii) instrument manuals and maintenance/service records should be available for technical personnel to review; (iii) all thermometers should be checked and calibrated against certified standard thermometers, and the information should be recorded in writing; and (iv) checked and calibrated thermometers should be in every refrigerator, freezer, incubator, water bath, heat block, etc., and temperatures should be recorded on each day of use. As specific requirements, (i) centrifuges should be calibrated for correct speed and the calibration should be recorded on the instrument, and (ii) all microscopes should be calibrated by using a stage and ocular micrometer, the calibrations should be posted on the instrument, and recalibrations should be performed if any ocular or objective is switched on that particular microscope. It is recommended that microscopes be recalibrated once each year, even if the oculars and objectives have not been changed. This is particularly true if the microscopes receive heavy use.

Reference Materials

Reference materials should be available for comparison with unknown organisms, refresher training, and the training of additional personnel. Ideal reference materials include formalin-preserved specimens of helminth eggs, larvae, and protozoan cysts; stained fecal smears of protozoan oocysts, cysts, and trophozoites; and positive blood smears. Color slides and atlases are recommended, although the level of microscopic focus cannot be changed. Reference books and manuals from a number of publishers are available, and selected ones should be part of the parasitology library. It is also recommended that you maintain a list of consultants who can be called in case questions arise.

Patient Outcome Measures

Patient outcome measures often refer to aspects of quality assurance that are monitored in addition to narrower parameters such as QC. Among the issues that are appropriate to monitor on an ongoing basis are the following:

1. Submission of the appropriate specimen within the correct time frame for collection
2. Condition of the specimen when received by the laboratory (too old, volume not sufficient, contains interfering substances, etc.)
3. Turnaround time for test results
4. Appropriateness of STAT requests
5. Improvements in compliance with established guidelines for the above, particularly after continuing-education efforts
6. Clinical relevance of and documented use of test results

All of these quality assurance parameters can be monitored by using The Joint Commission (formerly JCAHO) 10-step criteria for developing and implementing monitoring and evaluation activities (57; also http://www.jointcommission.org/accreditation/lab_standards_information.aspx [accessed 5/26/2015] and http://www.jointcommission.org/assets/1/18/2012_DSC_Cert_Guide.pdf [accessed 5/26/2015]). These 10 steps are listed below.

1. *Assign responsibility*. The designated individual identifies and assigns responsibilities to others within the department and ensures that these responsibilities are fulfilled.
2. *Delineate scope of care*. Delineating the scope of care involves identifying the diagnostic and thera-

peutic modalities used, times and locations where services are provided, types of personnel providing care, and all clinical services within the laboratory.

3. *Identify important aspects of care.* To effectively use the institution's resources in quality assurance, activities selected should be those with the greatest impact on patient care (the function occurs frequently or affects large numbers of patients, serious consequences could occur if services are not provided correctly or within set time frames, and the issue has tended to cause problems for staff and/or patients in the past).
4. *Identify indicators.* Indicators are well-defined, objective variables that are used to monitor the quality and appropriateness of any selected aspect of patient care. Within the laboratory, indicators could include clinical situations in which it may be inappropriate to use a particular test, situations in which inappropriate tests are often used, correct sequencing of tests, and clinical outcomes that may indicate inappropriate test use for a given situation.
5. *Establish thresholds for evaluation.* The thresholds for evaluation must relate specifically to the indicator and establish a value below which one does not continue to monitor and above which one continues to monitor and work on improvement and reaching the threshold or moving below.

   Note Some monitoring of an indicator usually occurs before a reasonable threshold can be identified. Once the threshold has been determined, monitoring and educational efforts continue, with the goal being to reach or go below the threshold. Once the threshold has been reached, consideration may be given to lowering it even more, thus leading to additional improvements in the indicator being monitored.

6. *Collect and organize data.* The following must be determined for each indicator: data sources, data collection method(s), sampling system, time frame for data collection, and process for comparing level of performance with set thresholds.
7. *Evaluate care.* If the data indicate that the acceptable threshold of performance has not been reached, extensive review occurs to identify problems or opportunities for improvement. An analysis of patterns or trends (specific shifts, units, personnel, skills) can help identify specific areas for improvement and changes. If the threshold of acceptance has been reached, it should be reevaluated in terms of keeping it where it is or possibly lowering it further to strive for additional improvements. If the decision is made to leave the threshold where it is, monitoring may be performed on a less frequent basis (every 6 months and then every year), just to verify that the data do not indicate that performance has declined.
8. *Take actions to solve identified problems.* The laboratory develops a plan of action that specifies who or what is expected to change, what action is appropriate, and when the changes should be complete. Three of the most common causes of problems are insufficient knowledge or poor communication, defects in the process, and poor compliance with process expectations. Frequently, educational presentations by various means (one on one, verbal presentations, written information, newsletters) can be used to help resolve the problems.
9. *Assess the actions and document improvement.* After sufficient time is allowed for improvements to occur, follow-up assessments are very important in documenting what progress has been made. This process must focus on the problem or opportunity for improvement, not on the action taken. If improvement is found, less frequent monitoring may be necessary; if the problem remains, new action should be taken with subsequent assessment for evidence of improvements.
10. *Communicate relevant information to the organization-wide quality assurance program.* It is critical that the findings not only be reviewed within the area being examined but also be conveyed and discussed at all levels of management. In addition to these discussions, documentation of such discussions is mandatory; one method often used is meeting minutes.

Continuous Quality Improvement, Total Quality Management, or 10-Step and FOCUS-PDCA for Performance Improvement Activities

Continuous quality improvement (CQI) (or total quality management) is a continuous quality improvement process that evaluates processes from a customer satisfaction point of view. The aim is continuous process improvement. This approach is a natural extension and expansion of the quality assurance programs that laboratories have been using for the past few years. CQI uses familiar tools such as check sheets, run charts, and flowcharts in new ways. Issues for review in a quality improvement program include some of the issues involved in a quality assurance program, such as timeliness of response to requests, turnaround time, and effective communication. *The problem-solving process in a CQI laboratory is done by broad-based teams with members from all affected groups inside and outside the labora-*

tory. Team members are trained in and use group process methods for identifying problems and generating solutions. Rather than management remaining "top down and autocratic," it becomes "bottom up and independent."

CQI laboratories focus on improving customer satisfaction; however, "customers" may be defined differently from the traditional use of the word. A CQI customer can be anyone who uses the products of the production process—a ward clerk responsible for charting results, a patient needing blood drawn for preadmission tests, or a physician waiting for a STAT result. Anyone who is an "end user" of the laboratory process is a customer and should be satisfied with the laboratory's product.

The Joint Commission uses the term "continuous quality improvement." The concept of "quality assurance" has been changed to "quality assessment and improvement" and includes the major trends leading to quality improvement. The formal surveillance process is deemphasized in lieu of promoting activities that better reflect the principles of CQI. *However, this does not suggest that the Joint Commission will abandon the 10-step process*. The use of CQI requires a shift in the organization's definition of quality from "good enough if it meets standards" to "we must work continuously to improve quality." This whole process is similar to any recommendation for problem solving; however, there are specific differences to consider.

1. Empowerment of employees to become involved in analyzing, operating, recommending change, and implementing solutions is necessary for CQI to be adopted.
2. CQI focuses on continuous monitoring of processes, not just limited issues.
3. The problems identified almost always cross over multiple departments or units.
4. The key issue always focuses on customer satisfaction and how we define it, measure it, monitor it for success, fix it when things go wrong, etc.
5. Groups who work on these identified problems include members from all areas relevant to the final service product (from initial ordering or product to final delivery).
6. The identification of areas for improvement originates from all levels within the institution.
7. A simplified version of the above is as follows.
   - A. Identify and choose a process to fix.
   - B. Analyze all aspects of the issue.
   - C. Select a possible solution.
   - D. Correct the process.
   - E. Monitor the results.
   - F. If results are satisfactory, solidify the change and move on to other issues.
   - G. Who does it? A group composed of representatives of all aspects of the process under review.

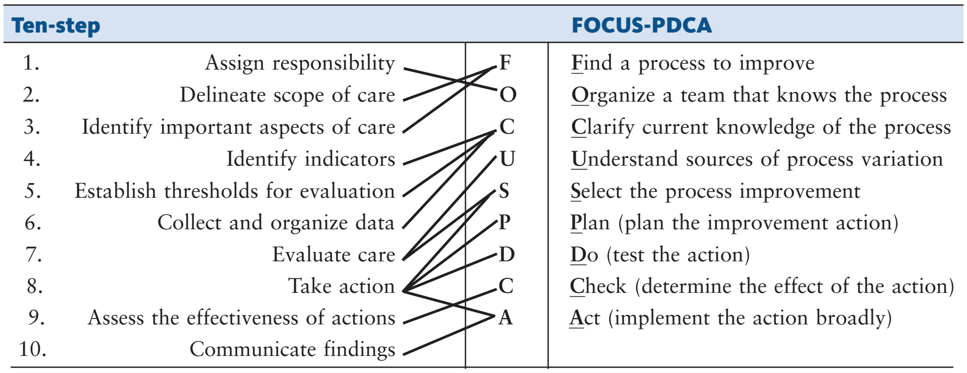
Another way to look at organizing performance improvement activities can be seen in Table 11.10. This approach merges the 10-step and FOCUS-PDCA approaches; similarities are certainly more numerous than differences.

CLIA '88 Inspection Process

HCFA began implementing the performance-based survey process in February 1996. This is a self-survey process, and the form used is titled the Alternate Quality Assessment Survey (AQAS). The form is designed to be used in certain laboratories for recertification purposes under the CLIA program in lieu of an on-site survey. Laboratories that were surveyed under the CLIA program via the State Agencies and found to have exceptional performance, i.e., no or few minor deficiencies and satisfactory proficiency testing, are potential candidates for the AQAS. The survey form contains questions that reflect an outcome-oriented, quality improvement type of assessment. Samples of some of the areas included in the questionnaire can be seen in Table 11.11.

Following receipt of the completed form, the State Agency reviews the laboratory's responses and supplemen-

TABLE 11.10 Ten-step and FOCUS-PDCA grid for organizing performance improvement activities



Ten-step			FOCUS-PDCA
1.	Assign responsibility	F	Find a process to improve
2.	Delineate scope of care	O	Organize a team that knows the process
3.	Identify important aspects of care	C	Clarify current knowledge of the process
4.	Identify indicators	U	Understand sources of process variation
5.	Establish thresholds for evaluation	S	Select the process improvement
6.	Collect and organize data	P	Plan (plan the improvement action)
7.	Evaluate care	D	Do (test the action)
8.	Take action	C	Check (determine the effect of the action)
9.	Assess the effectiveness of actions	A	Act (implement the action broadly)
10.	Communicate findings		

TABLE 11.11 Information regarding the HCFA self-survey process (CLIA): alternate quality assessment survey

Issue	Comments
Survey process	Respond to 45 “Yes/No” questions; some may require a written response, review dates, or submission of documentation. A checklist is provided to help you identify which questions require documentation. *Do not send original laboratory documents.*
“Review” questions	Questions that concern review of laboratory’s policies, procedures, records, etc., refer to a periodic review that is sufficient to demonstrate effective policies and to correct/modify ineffective ones. Reviews must be documented.
“Representative sample”	This means a review of a variety of records, requisitions, etc., that is sufficient to demonstrate whether policies and procedures are being accurately and completely followed.
Time line	The form must be completed and returned within 15 days of receipt to the State Agency return address.
General laboratory information	Includes name, address, type of CLIA certificate, levels of test complexity performed (waived, provider-performed microscopy, moderate, high), and annual test volumes (all areas of the laboratory). Microbiology includes bacteriology, mycobacteriology, mycology, parasitology, virology, general immunology, and syphilis serology. Parasitology includes direct preps, ova and parasite preps, and wet preps. Key information includes any changes regarding testing, testing sites, and laboratory personnel. *Documentation must include* demonstration of qualifications of newly hired personnel or changing positions since last CLIA on-site survey (licenses, diplomas, degrees, certificates, education, and experience).
Laboratory assessment	Specific areas within this category include patient test management, QC, proficiency testing, comparison of test results (different methodologies), relationship of patient information to test results, personnel assessment, communications and complaint investigations, and quality assurance review.
Attestation statement	The director of the laboratory is required to sign in ink to attest to the accuracy and honesty of the data submitted in the questionnaire.
Checklist	This checklist is designed to help you prepare and include written documentation to accompany the completed questionnaire.
Appendix A	This list contains a list of analytes or tests; if your laboratory performs any of these procedures, you must be enrolled in a proficiency testing (PT) program for those analytes or tests. *You must be enrolled in PT for the full extent of testing being performed (e.g., Gram stain, acid-fast stain, direct antigen testing, isolation, identification, and susceptibility).*
Appendix B	This section contains guidelines for counting tests (per questions regarding annual test volumes).

tal documentation. On the basis of this information, the laboratory may receive a telephone call requesting more information or may receive an on-site visit. If no problems are identified, the laboratory receives recertification for a 2-year period, provided that all relevant fees are paid. The certificate is forwarded to the laboratory. No laboratory will go longer than 4 years without an on-site survey. Approximately 5% of the laboratories are visited on-site to verify the effectiveness of this self-survey process.

The performance-based survey is a reward for exceptional performance and an incentive for improvement for those laboratories that have not yet been granted permission to use this self-survey approach. General information regarding the survey process can be obtained by calling Judith Yost (Director, Center for Laboratories, Health Standards and Quality Bureau) at HCFA [(410) 786-3531] (58).

New Quality Guidelines

Currently, there are ongoing efforts at both the national and international levels to provide guidelines for clinical laboratories in order to upgrade their QC and quality assurance programs to a broader definition—that of quality systems. Where QC and quality assurance programs monitor performance aspects of very specific operations, a quality system applies universal quality elements throughout the entire operation of the laboratory.

The application of Lean management systems to laboratory medicine is becoming more relevant. Lean is the term used to describe a principle-based CQI management system based on the Toyota production system that has been evolving for the past 70 years. Its origins were heavily influenced by the work of W. Edwards Deming and the scientific method that forms the basis of most quality management systems. Lean has two fundamental components: a systematic approach to process improvement by removing waste in order to maximize value for the end-user of the service and a commitment to respect, challenge, and develop the people who work within the system to create a culture of continuous improvement. Lean principles have been applied to many health care systems throughout the world to improve the quality and cost-effectiveness of services for patients. Also, a number of clinical/pathology laboratories have used Lean to shorten turnaround times, improve quality (reduce errors), and improve productivity. Increasingly, models designed to implement large-scale change in health care systems have evidence-based improvement methodologies (such as Lean CQI) as a core component (59).

ISO Guidelines

ISO (International Organization for Standardization) is the world's largest developer of standards. Since 2000, the worldwide standard for a quality system in business and industry has been the ISO 9001 guidelines (the fourth edition is published by the ISO). Countries trading products across international boundaries have found the standardized requirements very beneficial in improving quality; this approach has also reduced the time and cost of multiple inspections due to the international nature of their business. In the future, there will be a universal standard for quality management in medical laboratories: ISO 15189 (60, 61). ISO 9001 Quality Management Systems is under review and an updated version is expected by the end of 2015. All ISO standards are reviewed every 5 years to make sure they remain as helpful and relevant (http://www.iso.org, accessed 4/3/2015).

When the large majority of products or services in a particular business or industry sector conform to International Standards, a state of industry-wide standardization can be said to exist. This standardization is achieved through consensus agreements between national delegations representing all the economic stakeholders concerned: suppliers, users, government regulators, and other interest groups, such as consumers. They agree on specifications and criteria to be applied consistently in the classification of materials, in the manufacture and supply of products, in testing and analysis, in terminology, and in the provision of services.

According to their objectives, laboratories will choose a recognition of their quality management system with an ISO 9001 certification or a recognition extended to the technical skills with an ISO 17025 or ISO 15189 accreditation. The contents of these last two documents are very similar, and both integrate requirements of the standard ISO 9001. The standard ISO 17025 is somewhat distant from clinical laboratory needs, requiring many efforts of adaptation, just like the ISO 9001 standard. The standard ISO 15189 seems to be well adapted but more constraining, considering the detailed requirements required. It necessitates a perfect control of the preanalytical phase, which is difficult to acquire in a clinical framework where the clinical specimens are not taken by the laboratory staff.

A number of advantages are associated with the use of ISO. The focus on patients has been reestablished, all processes are identified and subject to continuous improvement, and performance measurements provide an integrated picture of results. Measurements subsequently lead to improvement of quality of care and to quality system improvements. The documentation system can serve the organization's needs without leading to bureaucracy. Given the need for adequate quality management tools in health care and the need to demonstrate quality, the positive effects suggest that ISO will become more prevalent in health care organizations (62).

ISO provides technical and quality management, which results in benefits observed in daily laboratory practices. Technical requirements can include the addition of formal personnel training plans and detailed records, method development and validation procedures, measurement of method uncertainty, and a defined equipment calibration and maintenance program. Also, an expanded definition of the sample preparation process can be implemented and documented to maintain consistency in sampling. Management quality improvements often emphasize document control to maintain consistent analytical processes, improved monitoring of supplier performance, a periodic contract review process for documenting customer requirements, and a system for handling customer comments and complaints. Continuous improvement is monitored and improved through corrective and preventive action procedures and audits. Quarterly management review of corrective actions, nonconforming testing, and proficiency testing results also identifies more long-term trends. The practical benefits of these technical and management quality improvements can be seen on a daily basis in the laboratory. Faster identification and resolution of issues regarding methods, personnel or equipment, improved customer satisfaction, and overall increased laboratory business are all the result of implementing an effective quality system. Certainly, the ISO system provides one option among various quality improvement approaches (63).

CLSI (NCCLS) Model

In the 1990s, blood centers and transfusion services within the United States began adopting the concepts of quality systems into their routine operations, primarily based on direction provided by a 1993 FDAQA guideline (64). After a series of revisions, the American Association of Blood Banks (AABB) published its 10 Quality System Essentials (QSEs) (65). These have been incorporated into the most recent edition of the AABB *Standards for Blood Banks and Transfusion Services* (66).

It became very obvious that the QSEs proposed for the blood bank were applicable to the entire laboratory operation. These essentials also incorporated many of the quality requirements developed by CLIA '88, The Joint Commission (formerly JCAHO), and the College of American Pathologists (CAP).

Recognizing that standardization would be not only valuable, but also essential for high-quality laboratory operations as a whole, the Clinical and Laboratory Standards Institute (CLSI) (formerly known as the National Committee for Clinical Laboratory Standards [NCCLS]) developed a subcommittee to prepare a guideline encompassing a complete quality system for the clinical laboratory. This document was recently published as QMS01-A4 (GP26-A4), *Quality Management System: A Model for Laboratory Services; Approved Guideline—Fourth Edition* (67).

Factors Influencing Future Laboratory Practice

The current atmosphere of uncertainty within the health care environment in the United States in the second decade of the 21st century is well recognized. Funding and support for health care are diminishing, and the economic future of health care is hostage to a number of identifiable trends and concerns (Table 11.12).

As consolidation among payers continues through mergers, acquisitions, and other arrangements, providers of care find themselves with fewer buyers of care with whom to negotiate. This requires the development of strategies through which provider leverage can be maintained. Consolidation on the provider side has been one response. Price reductions and exclusive arrangements have been others. Response to these changes also includes affiliation with community providers to increase bargaining strength and aggressive cost reduction to allow more competitive bidding. However, many institutions are now facing the acceptance of marked reductions in reimbursement rates or terminating contracts. As additional changes occur and negotiations continue on these issues, each institution much achieve a balance between the market share realized from each contract, the revenue per service unit, and the total revenue.

TABLE 11.12 Factors influencing future laboratory practice

- Growing federal deficit
- Concerns about future viability of Medicare and Medicaid
- Increased inability of states to fund Medicaid costs
- Expanding potential of molecular technologies to detect potential for disease at the genetic level prior to the display of symptoms
- Health care insurance and delivery system to end users complicated by problems related to access, quality, safety, and confidentiality
- Increases in health care costs due to aging population and growth of expensive technology
- A highly adversarial climate of blame and controversy among the various players in the health care arena: insurers, drug companies, technology companies, patients, the general public, health care providers, lawyers, politicians, and economists; all of these groups tend to support conflicting solutions/positions. Significant modification of the managed-care system has already occurred; however, there is little consensus about other viable options. The Affordable Care Act, which was passed during the first term of the Obama administration, contains provisions that radically alter all aspects of health care delivery and financing. Whatever the ultimate fate of the Affordable Care Act, shrinking financial resources available for health care will remain the norm. Thus, reduced funding will continue to impact health care providers and lead to increasing conflict over the allocation of resources.

[a] Adapted from reference 4.

Marketplace trends include continued growth in managed care, particularly in the health maintenance organization sector; integration of payers, providers, suppliers, and buyers; mergers and alliances; integration between hospitals and physicians; continued pressure for provider consolidation to address excess bed capacity; continued shift from inpatient to outpatient procedures; and provider alliances based on complementing services (linkage of primary-care facility with the tertiary or quaternary expertise of another institution).

Strategies for survival will include (i) increased ability to capture market share, preferably related to covered lives rather than patient days; (ii) improved information systems to accurately identify costs (fixed, variable by service, procedure) and track the profitability of contracts; and (iii) recognition of the importance of these issues and implementation of proactive measures to structure the organization for survival in the coming years.

Managed Care

With the increase in health care contracts and overall health care reform, a number of potential issues will affect the laboratory of the future. Each institution is actively seeking new markets, with the overall objective being to increase the patient base served by that particular institution. As these contracts increase in number, the reimbursement approach becomes one of capitated payment; the provider takes on the risk previously assumed by the third-party payer. The provider receives a certain amount for each patient per year, the assumption being that the care can be delivered at less than the reimbursement capitated amount; thus, a profit is realized. As these changes occur, there continue to be mergers and the development of large care networks. However, as more large networks are developed, there are fewer contracts on which to bid. Each institution may be forced to consider contracts that pay less than the current costs of service. Obviously, these changes serve as a tremendous incentive to deliver care at continued reduced costs. Once contracts are lost to other bidders, it may be more difficult to recapture that lost market.

Managed care is changing from a cost containment environment to a value purchasing environment. Considerations include careful examination of what is being received for the money spent, increases in requests for outcome data, and case management options. As competitiveness in the managed-care arena increases, long-standing partnerships between institutions and health maintenance organizations will have no inherent merit in guaranteeing continued partnerships. More focused medical management, lower costs, and higher efficiency will determine the continuation or elimination of present agreements. The new environment will demand increased cohesiveness, cooperation, and creativity on the part of all employees within an institution.

An insurance, payment, and delivery system for health care, loosely described as "managed care," is increasingly viewed as dysfunctional by consumers (i.e., patients), providers, and employers, the last of which constitute the primary source of funding for health insurance for the employed sector of the population. In the current environment of managed-care dominance within the United States, clinical laboratories continue to change their focus of operations and mission in response to the continually changing landscape. Traditional laboratories that are unwilling to change and adapt to this environment will probably not survive. A synopsis of changes since the early 1990s is provided in Table 11.13.

There appears to be a climate of blame in which the various players in the health care arena reproach each other and advance conflicting solutions. Although the "demise" or significant modification of the managed-care system is widely predicted, there is a distinct absence of any consensus about the parameters of politically or economically viable alternatives. Nonetheless, it appears that "something else" will emerge—a return of the "managed-competition" plan proposed in the early years of the Clinton presidency, a national health model resembling the Canadian system, implicit and explicit rationing of resources, or other combinations and approaches. The Affordable Care Act, which was passed during the first term of the Obama administration, contains provisions that radically alter all aspects of health care delivery and financing. However, implementation has only begun, and it is clear, that whatever the outcome of the Affordable Care Act, decreased financial resources available for health care will continue.

TABLE 11.13 Evolution of the health care delivery system and impact on clinical laboratory testing[a]

Cause or development	Changes
Failure to slow exponential growth of total health care expenditures	Rapid and progressive penetration of MCOs in early 1990s; diagnostic testing viewed as a "commodity" rather than medical service
Growth of managed care	Hospital-based laboratories in a financial downward spiral
Shorter LOS; reduction in admissions	Reduction in in-house testing
Managed-care-associated businesses, consolidation and networking; outreach test market, point-of-care and satellite testing	Major strategies developed for restoring tests eliminated by massive changes
Regulatory restrictions imposed by CLIA '88; expanded penetration of MCOs (reimbursement limited to a few in-house procedures)	POLs closing
Reference laboratories gaining more tests through contracting with MCOs	Continued reduction of test volumes in POLs and hospital laboratories

[a] CLIA '88, Clinical Laboratory Improvement Amendments of 1988; LOS, length of stay; MCO, managed-care organization; POL, physician office laboratory.

Financial Considerations

Capitated reimbursement has already been mentioned as one of the driving forces behind the necessity to deliver health care at continuing reduced rates. Each year, reimbursement rates decline; currently, leverage lies with the payer, not the provider. Competitive bidding has become an absolute necessity. Also, institutions continue to review their costs in much greater detail than at any time in the past. To survive, each institution must know exact costs so that it can bid appropriately and remain financially viable. Complicating this approach are the various regulatory requirements and the fact that each contract is somewhat different in terms of services and costs. Laboratories are continually reviewing their test menus, methodologies, etc., for the most clinically relevant, cost-effective approach to diagnostic testing.

Current problems related to health care costs include a growing federal deficit and concerns about the future viability of Medicare, the increasing inability of state budgets to fund rising Medicaid expenditures, an aging population that will generate increasing demands on health care resources, the proliferation of costly technological and pharmacological advances in the ability to diagnose and treat disease, and a health care insurance and delivery system unable to provide insurance and predictable access to care for approximately 40 million Americans (3).

Current Regulations

Immediately prior to the 21st century, Congress began to focus on legislation and regulation in health care, perhaps the two most striking examples of which are the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and the Health Insurance Portability and Accountability Act of 1996 (HIPAA). The four main regulations that are relevant to pathology and laboratory medicine are CLIA, HIPAA, the Occupational Safety and Health Administration (OSHA) standards for occupational exposure to blood-borne pathogens, and the Stark regulations that prohibit self-referral by health care providers.

CLIA '88

The Clinical Laboratory Improvement Amendments were enacted in 1988; however, the regulations were not implemented until 1992. Although changes to the regulations continue to be made, the amendments in their current form are well established and are applicable to all testing performed on humans. Specific regulations include personnel standards, patient test management, quality assurance, proficiency testing, provisions for inspection and certification, and both civil and criminal penalties for noncompliance. Inspection and accreditation options are multiple and include government and private-sector organizations such as the College of American Pathologists and the Joint Commission.

HIPAA

The Health Insurance Portability and Accountability Act was passed in 1996. The Privacy Rule standards address the use and disclosure of individuals' health information (protected health information) by organizations subject to the Privacy Rule (covered entities) as well as standards for individuals' privacy rights to understand and control how their health information is used. Within the U.S. Department of Health and Human Services, the Office for Civil Rights (OCR) has responsibility for implementing and enforcing the Privacy Rule with respect to voluntary compliance activities and civil money penalties (http://www.hhs.gov/ocr/privacy/hipaa/understanding/summary/index.html; accessed 4/3/2015).

A major goal of the Privacy Rule is to assure that individuals' health information is properly protected while allowing the flow of health information needed to provide and promote high quality health care and to protect the public's health. The Rule strikes a balance that permits important uses of information, while protecting the privacy of people who seek care and healing. Given that the health care marketplace is diverse, the Rule is designed to be flexible and comprehensive to cover the variety of uses and disclosures that need to be addressed.

"Individually identifiable health information" is information, including demographic data, that relates to and that identifies the individual or for which there is a reasonable basis to believe it can be used to identify the individual:

- the individual's past, present, or future physical or mental health or condition,
- the provision of health care to the individual, or
- the past, present, or future payment for the provision of health care to the individual.

OSHA Standards for Occupational Exposure to Blood-Borne Pathogens

The standards for exposure to blood-borne pathogens were developed in 1991 by OSHA of the Department of Labor in response to widespread concerns by unions representing health and hospital workers concerning the inadequacy of protective practices, equipment, and other safeguards for employees with regular and predictable exposure to body fluids and substances with the potential to transmit HIV, hepatitis B virus, and hepatitis C virus. More complete information can be found at http://www.osha.gov/SLTC/bloodbornepathogens/index.html (accessed 4/3/2015).

In order to reduce or eliminate the hazards of occupational exposure to blood-borne pathogens, an employer must implement an exposure control plan for the worksite with details on employee protection measures. The plan must also describe how an employer will use a combination of engineering and work practice controls, ensure the use of personal protective clothing and equipment, and provide training, medical surveillance, hepatitis B vaccinations, and signs and labels, among other provisions. Engineering controls are the primary means of eliminating or minimizing employee exposure and include the use of safer medical devices, such as needleless devices, shielded needle devices, and plastic capillary tubes.

The Stark Laws

The Stark regulations were enacted sequentially over a period of several years and severely limit referral of a wide variety of services (including laboratory testing) to entities in which the referring provider has a financial stake. The regulations are named for Rep. Fortney "Pete" Stark (D.-CA) who was the legislative sponsor of the initial regulations enacted in 1992 (Stark I) that prohibited a physician from making Medicare referrals for clinical laboratory services if the physician or an immediate family member had any financial relationship with the laboratory. In 1993, the Stark II legislation extended the regulations to Medicaid and broadened coverage to 10 additional health services, including, among others, occupational and physical therapy, durable medical equipment, radiology, and radiation therapy. However, the regulations are very complex and difficult to interpret. Enforcement by the government, particularly the Justice Department, is closely linked to other "fraud and abuse" enforcements under a variety of federal anti-kickback and "false claims" statutes (68).

Decentralized Testing

In the last few years, many inpatient procedures have been shifted to the outpatient setting in response to payer per diem cost containment initiatives. Most institutions are actively involved in developing or expanding outreach programs, including laboratory services, teaching, and consultations. Another area that will continue to expand is point-of-care testing (POCT), or testing at the site of the patient (bedside, emergency room, or clinic), or alternative-site testing (home or shopping mall). With this expansion comes new technology that is advertised as "fail-safe and foolproof," some of which may be true and some of which may be exaggerated. Obviously, this approach is directly linked to patient and overall customer satisfaction related to a short turnaround time for test results. Since more and more patient care is being delivered on an outpatient basis as decentralized care, reductions in length of hospital stay and in cost continue to be issues for review.

In some institutions that service very specific patient groups, POCT may offer distinct advantages; however, in institutions with highly efficient specimen transport systems and rapid response capabilities within the main clinical laboratory, advantages of POCT may be minimal to none. Continued review of regulatory requirements, QC issues, proper and consistent documentation, proficiency testing, performance enhancement, and cost-effectiveness is mandatory for success. As technology moves toward microcomputerization, microchemistry, chip technology, and enhanced test menus, the use of POCT will require ongoing scrutiny (69, 70).

Although the push for decentralized testing may be coming from a variety of sources, it is generally thought that the central laboratory has become more involved in managing the new testing technologies and trying to circumvent anticipated problems with testing quality and patient care.

There are real, continued concerns about test accuracy, precision, training, and QC issues; personnel training and experience; and proficiency testing by those actually performing the testing. To date, there is little scientific evidence for cost reduction and/or reduction in length of stay. There appears to be mounting evidence that in some settings, the cost is higher than that of central laboratory testing.

Laboratory Services

Large, integrated testing laboratories may become "the laboratory of the future," particularly considering the issue of economy of scale; i.e., the more we perform large numbers of the same test, theoretically the lower the cost. Although this approach is more applicable to some areas of the laboratory (chemistry), it is being carefully evaluated even in labor-intensive areas with less automation (microbiology). As technology continues to change, the departmental limits within any laboratory (chemistry, hematology, microbiology, or blood bank) continue to become less well defined. Many laboratory operations are now structured around automated and manual methods rather than specific clinical disciplines. This approach will continue and will also affect personnel training and utilization. Review of in-house versus send-out tests will continue, particularly regarding cost containment parameters.

With continued development and implementation of computerized history algorithms, clinical pathways, and case management, more structured support for clinical decision making will also dramatically affect the laboratory. Inappropriate test-ordering patterns and overutilization will become less and less significant. The use of algorithms may also become much more common within the laboratory itself. All of these changes will be developed through the use of multidisciplinary teams, including all members of the health care delivery system (ancillary departments, nursing staff, and physicians).

New technology will continue to be evaluated for a number of parameters, including type of data, technical characteristics, diagnostic characteristics, clinical utility, and cost-benefit analysis. Laboratory design will be another key issue and will be influenced by a number of factors, including consolidation of health care facilities; continued shift to outpatient care and POCT and alternative-site testing; development and use of clinical paths; and development and evaluation of new technologies. Physical plant design should consider a number of factors, such as analysis of institution mission, scope of the project, and specific objectives; written statements of needs; mechanisms for keeping people informed; careful analysis of operational needs (work flow, traffic patterns, etc.); detailed review of current and future space requirements (consolidation of instrumentation, workstations, storage capacity, and equipment location); flexibility of design (modular furniture and utilities); and safety issues.

Technological Trends

Currently, hospitals and clinics are not able to meet their expenses without continued decreases in labor costs. During the next few years, the greatest savings in laboratory costs will come from technology that leads to labor reductions. Laboratory automation has the potential to be a highly successful, cost-saving measure and has been implemented in Japan, where many laboratories employ 5 to 10 times fewer full-time equivalents than European and North American laboratories while still achieving similar productivity results. Future expectations include the performance of all but a few esoteric tests by automated systems, the use of mass spectrometry, and performance of most routine testing on a single automated analyzer. It is very likely that specimens will arrive in the laboratory ready for testing, with all processing having been done prior to receipt by the laboratory. The specimen may contain information chips carrying patient medical history and physiologic data at the time of sampling. These chips may also allow wireless encoding and reading of specimen information. Thus, the old specimen-processing area can concentrate on customer service, provision of medical information, and client education.

As an adjunct to the highly automated core laboratory, miniature analyzers and POCT will play a major role in testing. Multianalyte, spectroscopy-based, noninvasive sensors will provide a wide range of tests at the bedside; there is even the possibility of a wireless invasive analyzer that would reside in a device that could be injected or implanted under the skin or elsewhere in the body.

New molecular tools have been developed rapidly, especially with the excitement over the completion of the human genome project. Synthetic antibody-like molecules with high affinity and specificity have been developed from both DNA and RNA. These molecules may replace antibodies in molecular diagnostics, achieving tremendous levels of sensitivity.

Biometrics is the use of sensors to measure natural body features for positive identification. In the future, biometrics will be extremely helpful in confirming that patients and their body fluids or other specimens are correctly identified. A patient's complete medical history may be available on the Web when the patient simply places a finger into the sensor mechanism.

Nonlaboratory systems, such as robots, will be used much more commonly for delivery functions, saving a significant amount of money as well as improving service. Surgery robots that can perform complex procedures through very small incisions are currently under development, and some have been implemented.

An area of research and laboratory practice that will be critically impacted by complex and interrelated legislative, regulatory, and administrative developments is stem cell research and the application of cloning technology to medical practice in tissue and organ transplantation and reproductive technology. The cloning research issue is closely linked to the highly emotional controversy over abortion and genetic manipulation and has elicited strong and conflicting opinions from various constituencies, including the research and medical community, religious groups, and organizations committed to research and treatment of specific diseases.

Clinical Decision Support

A number of approaches are being developed and used to serve as "practice guidelines"—more structured approaches for both information gathering and clinical decision making related to the patient. A clinical pathway is defined as a set of interventions or actions that is used to help a patient move progressively through a clinical experience to an expected, positive outcome. One of the main reasons for developing clinical pathways is to provide a tool to evaluate clinical patient management and outcomes. Once defined sets of expectations are developed, data can be used to monitor variances. The cost of care and utilization of resources can then be monitored more closely.

By using clinical pathways for predictable clinical outcomes (conditions, diseases, and procedures selected are generally very well established and patient outcomes are consistent), many also believe that patient care is more consistent. These clinical pathways can also be used as a teaching tool for all health care personnel. The development of clinical paths must be a multidisciplinary effort, with input from all relevant members of the health care team. Many institutions are also using the process of clinical path development to foster team building and continuous quality improvement initiatives.

Regardless of names or titles, the following are becoming more widely used and accepted. They are listed from the most well-defined and predictable medical situations (clinical pathway) to very complex cases with many underlying factors and complications (case management).

1. Clinical pathways (care maps) (used for conditions, diseases, and procedures for which the patient outcome is very predictable)
2. Clinical guidelines (used for care situations in which the outcome may have a few more variables)
3. Clinical algorithms (used for patient outcome situations in which more options may be required)
4. Case management (generally used for very complex cases, for which it is very difficult to establish a predetermined pathway; the patient may have multiple medical problems and/or complications)

Personnel Issues

Many personnel issues will continue to be relevant and important. Particular emphasis will probably include (i) the use of unlicensed individuals in relationship to CLIA '88 and various states developing, implementing, and revising personnel regulations; (ii) cross-training and the use of multiskilled laboratory personnel; (iii) tremendous flexibility in covering fluctuations in workload; (iv) expansion of the use of multidisciplinary teams; (v) continued emphasis on horizontal rather than vertical management structures; and (vi) development and utilization of personnel competency assessment programs (now mandated by regulations).

Changing Demographics

The aging of the population will have a profound effect on many health care issues. Laboratory testing and monitoring will be no exception. Patient access, alternative-site testing, and clinical decision support options will play a large role in helping manage this expanding population.

Emerging Diseases

Not only do we have to deal with expanding diseases and conditions in the elderly, but also we now are faced with some serious problems related to infectious-disease threats. There is explosive population growth worldwide with expanding poverty and urban migration; international travel is increasing; and technology is rapidly changing. All of these factors affect our risk of exposure to the infectious agents in our environment. In recent years, our antimicrobial drugs have become less effective against many infectious agents, and experts in infectious diseases are concerned about the possibility of a "postantibiotic era." As a result, our ability to detect, contain, and prevent emerging infectious diseases is in jeopardy.

Bioterrorism

In the wake of the events of September 11, 2001, credible threats of terrorism that include the potential use of nuclear, chemical, and biological agents have had significant impacts on the funding and costs of providing laboratory services. The identification of clinical cases and environmental contamination caused by deliberate dissemination of *Bacillus anthracis* through the mail system during the early part of 2002 emphasized this lesson for a number of microbiology laboratories. These events and the fear of additional terroristic use of other infectious agents have not only alarmed the public, the government, and the medical and microbiological community but also focused attention on technical capabilities, biologic safety infrastructure, and staff preparation (71, 72).

Unfunded programs will impact training costs, acquisition and deployment of equipment, facility modification, and other unfunded mandates, such as the CDC-issued rules (August 2002) requiring all laboratories to report to the government whether they are in possession of "select agents" that could be used in a bioterrorist attack. The rule is available at http://www.selectagents.gov/regulations.html (accessed 5/26/2015) (4). However, increased federal concerns about laboratory preparedness have generated some increased funding for specific areas of the laboratory; it is more likely that in the event of actual or suspected biological attacks, the impact on the laboratory will be to significantly increase laboratory services. These increases will focus on unusual/potentially dangerous diagnostic challenges and on the potential massive increase in demand for routine laboratory services required for the care of large numbers of acutely ill patients.

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12

Medical Parasitology: Case Histories

Protozoal infections

Helminth infections

Blood parasite infections

Diagnostic methods and proficiency testing

The case histories in this chapter are taken from actual practice and provide an excellent method of review for the reader. Each case includes the history, laboratory findings, and clinical course. Self-assessment questions and a comprehensive discussion supplement each history. Also, parasite images photographed from actual bench work microscopy accompany each case. The cases illustrate many kinds of potential diagnostic problems encountered within the parasitology section of the laboratory and emphasize clinical relevance and recommended approaches for specimen ordering, collection, processing, testing, and reporting. Although not every parasite known to be a human pathogen is included, a wide spectrum of organisms and diseases is presented to illustrate particular points.

Protozoal Infections

Case 1

A 30-year-old patient complained that he had had diarrhea for about a week, and specimens were submitted to the laboratory. He had previously been in excellent health with no complaints, although he did report an unexplained weight loss of several pounds. On examination of the permanent stained smears, the images in Fig. 12.1 were seen. These protozoan trophozoites measured approximately 15 μm long, and the precyst measured 16.5 μm long. The permanent stained smears have been stained using Wheatley's trichrome stain modification. The test result is reported as follows: "*Entamoeba histolytica* trophozoites present."

Questions

1. Based on the diagnosis of diarrhea and the laboratory findings, how would you identify the objects seen in the permanent stained smears?
2. Was the report correct as submitted to the physician? Why or why not?
3. Is diarrhea a normal finding in an infection caused by this organism?
4. Why is it important to report the presence of this organism correctly?

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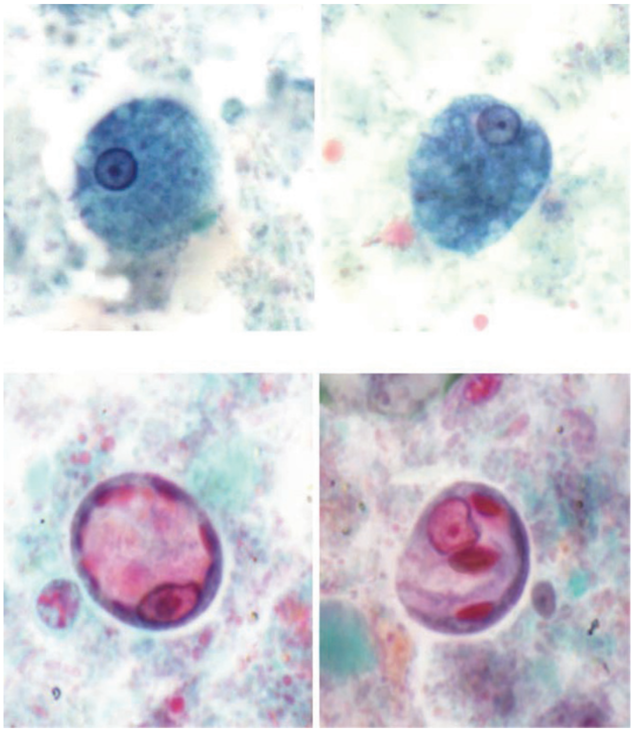

Figure 12.1 Case 1. Microscopic images (oil immersion 100× objective); permanent stained smear. doi:10.1128/9781555819002.ch12.f1

Correct Identification

The images presented in this case history are trophozoites of *Entamoeba histolytica/E. dispar*; therefore, the physician's report ("*Entamoeba histolytica* trophozoites present") was incorrect. You will notice that the amebic trophozoites in Fig. 12.1 did not contain any ingested red blood cells (RBCs) within the cytoplasm. If no RBCs are seen in the cytoplasm and the trophozoites measure greater than or equal to 12 µm, the true pathogen, *E. histolytica*, cannot be differentiated from the nonpathogenic *E. dispar* or other nonpathogens. Currently, there are immunoassays available that can confirm the presence of organisms in the *E. histolytica/E. dispar* group, as well as one immunoassay that can confirm the presence of the pathogen *E. histolytica* (1–5). Note that the precysts contain one nucleus and chromatoidal bars with rounded ends, also confirmation of the *E. histolytica/ E. dispar* group (precysts of *Entamoeba coli* would tend to have two nuclei; chromatoidal bars would rarely be present and would have sharp ends.

The correct laboratory report for the organisms seen in the case history would have been "*Entamoeba histolytica/E. dispar* trophozoites present." A computer comment could be added as follows: "Based on organism morphology, the presence of the true pathogen, *Entamoeba histolytica* (cause of amebiasis), could not be confirmed."

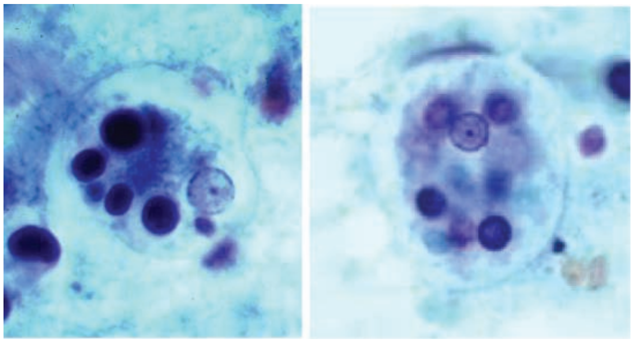

Figure 12.2 Case 1. *Entamoeba histolytica* trophozoites containing ingested RBCs. doi:10.1128/9781555819002.ch12.f2

Trophozoites of *E. histolytica*, the true pathogen, can be seen in Fig. 12.2; the correct identification is confirmed by the presence of ingested RBCs within the organism cytoplasm.

If your laboratory offers the immunoassay specific for the true pathogen *E. histolytica*, the computer comment could be: "If you want confirmation of the presence of *Entamoeba histolytica*, submit a fresh stool sample." The immunoassays for *E. histolytica* or the *E. histolytica/E. dispar* group require fresh or frozen stools; preserved specimens are not acceptable for testing.

Prior to the separation of *E. histolytica* into two distinct species (*E. histolytica* and *E. dispar*), there were suggestions that some *E. histolytica* strains were pathogenic and some were not. Now that *E. dispar* has been classified as a nonpathogenic organism, totally separated from the pathogenic *E. histolytica*, treatment of *E. dispar* infection is usually not recommended. However, this recommendation is based on the separation of the two species by molecular means, not by morphology alone. Since few laboratories will be routinely identifying the two *Entamoeba* species to the level of *E. histolytica* or *E. dispar*, the clinician will have to decide on the basis of clinical findings whether the patient has true pathogenic *E. histolytica* or nonpathogenic *E. dispar* infection. The laboratory report will merely indicate the presence of the "*Entamoeba histolytica/E. dispar*" group.

Infection with *E. dispar* cannot be diagnosed on the basis of morphologic criteria alone, even from the permanent stained smear. When the organism is seen in the routine ova and parasite examination, it cannot be differentiated from *E. histolytica* unless trophozoites are seen to contain RBCs in the cytoplasm, a finding that is diagnostic for *E. histolytica*. When routine diagnostic methods and not immunoassay procedures specific for *E. histolytica* are used, morphologic criteria would provide characteristics of both; the laboratory report would indicate "*Entamoeba histolytica/E. dispar* group."

Key Points for Laboratory Identification

1. When routine ova and parasite (O&P) examinations are ordered, three specimens (stool) should be submitted for the diagnosis of intestinal amebiasis.
2. Any examination for parasites in stool specimens must include the use of a permanent stained smear (even on formed stool).
3. Presumptive identification on a wet preparation must be confirmed by using the permanent stained smear. Without using *E. histolytica*-specific antigen detection tests, the presence of the organism(s) should be reported as follows. Trophozoites containing ingested RBCs within the cytoplasm should be reported as the true pathogen ("*Entamoeba histolytica* trophozoites present"). However, if precysts, cysts or trophozoites containing no ingested RBCs are seen, the report should indicate that the *Entamoeba* group is present ("*Entamoeba histolytica*/*E. dispar* group trophozoites and cysts present").
4. Six smears should be prepared at sigmoidoscopy but should not take the place of the O&P examination.
5. Immunoassay procedures can be performed to confirm the presence of the *E. histolytica*/*E. dispar* group or can be used to differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*. Remember that some of these reagent kits may require fresh or frozen stools only (Cary-Blair may also be an option—check with manufacturer); formalin-preserved stool or stool containing polyvinyl alcohol is not acceptable.
6. The serologic test for antibody may *or may not* be positive in intestinal disease and is much more likely to be positive in extraintestinal disease in amebiasis (*E. histolytica*).

Case 2

A 28-year-old man presented with rhinosinusitis and was diagnosed as having a squamous cell carcinoma of the nasal septum. He was presumed to be a healthy and immunocompetent individual; however, he was later diagnosed with AIDS. As the diagnostic workup was expanded and other possible etiologic agents were considered, the images in Fig. 12.3 were seen from agar culture plates.

These organisms have spine-like pseudopods, with the average diameter of the trophozoites being 30 μm. The nucleus has the typical large karyosome, and morphology can be seen on a wet preparation. The cysts are round with a single nucleus, which also has the large karyosome seen in the trophozoite nucleus. The double wall is usually visible, with the slightly wrinkled outer cyst wall and what has been described as a polyhedral inner cyst wall (2, 3, 6).

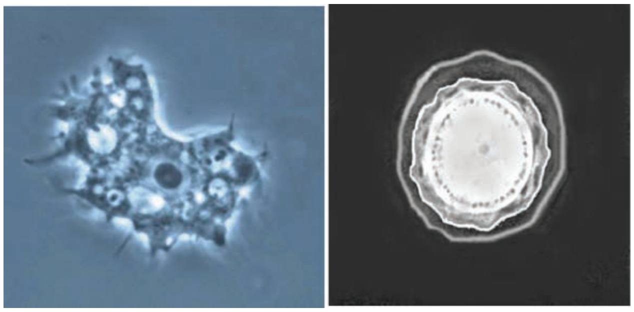

Figure 12.3 Case 2. Protozoan. (Left) Trophozoite with "spiky" acanthapodia; (right) cyst with hexagonal double wall. doi:10.1128/9781555819002.ch12.f3

Questions

1. Can you describe the type of culture system that was used to support the growth of these organisms?
2. What do you think these organisms are (genus)? Why?
3. What morphologic characteristics support your identification?
4. What impact would AIDS have on this opportunistic infection?

Correct Identification

Assuming the possibility of infection with free-living amebae, diagnostic procedures could include agar plate cultures for the recovery of trophozoites and cysts of *Acanthamoeba*. Nonnutrient agar plates are routinely used with a bacterial overlay. The organisms are *Acanthamoeba* spp. Note the spiky acanthapodia in the trophozoite stage and the presence of the characteristic double-walled cyst form.

The protracted course in this case is characteristic of severe illness caused by *Acanthamoeba* and distinguishes this disease from primary amebic meningoencephalitis (PAM), caused by *Naegleria*, which has a much more acute presentation. Additional features that helped to distinguish the patient's *Acanthamoeba* infection from that caused by *Naegleria* include the typical trophozoite and cyst morphology obtained from the culture system.

Although this patient was diagnosed as having a squamous cell carcinoma of the nasal septum, this was an incorrect diagnosis. Once the diagnosis of AIDS was made, the workup was expanded to include possible parasitic pathogens, particularly organisms in the free-living amebae group. The final diagnosis was AIDS along with disseminated acanthamoebiasis. This case points up the importance of considering all possibilities, particularly in a patient who appears to be immunocompetent but who may actually be immunocompromised for one or more reasons.

An early diagnosis can be made if amebic infection is considered in patients with various presentations. *Acanthamoeba* spp. are now well accepted as opportunistic pathogens in immunocompromised patients with AIDS, generally those with a low CD4$^+$ cell count. Unfortunately, diagnosis of this infection requires a high index of suspicion, since both clinical and histologic findings may mimic those of disseminated fungal or algal disease or noninfectious causes such as carcinoma. Patients with AIDS and *Acanthamoeba* infection may experience general symptoms such as fever and chills, nasal congestion or lesions, neurologic symptoms, and musculoskeletal and cutaneous lesions. Some patients, especially those with AIDS, develop erythematous nodules, chronic ulcerative skin lesions, or abscesses.

The primary site of infection is thought to be the sinuses and lungs. The skin is also thought to be a possible portal of entry. In patients with disseminated disease, the duration of infection from onset to death can range from 7 days to 5 months. Since early therapy can alter the clinical outcome, recognition of this disease is critical. It is important to initiate appropriate diagnostic testing for confirmation of the causative agent as soon as possible.

Acanthamoeba spp. have been found in the upper respiratory tract in humans with and without symptoms. In cases of disseminated disease, the portal of entry remains unknown but is suspected to be the respiratory tract. However, in patients with skin lesions, the skin may be the site of primary inoculation. Although many of the reported cases have been seen in compromised patients, cases have also occurred in immunocompetent hosts.

Although *Naegleria* dissemination outside the central nervous system (CNS) may occur, it is apparently rare and is unlike the protracted situation seen in this patient. The morphology of *Naegleria* trophozoites is quite different from that of *Acanthamoeba* trophozoites, and an image can be seen as in Fig. 12.4. Note the lobular pseudopodia as compared with the "spiky" pseudopodia seen in *Acanthamoeba* trophozoites (Fig. 12.3).

Figure 12.4 Case 3. Protozoan. (Left) Trophozoites growing on a nonnutrient agar plate seeded with *Escherichia coli* bacteria; (right) stained trophozoite (note the more globular pseudopods compared with the organism in case 2).
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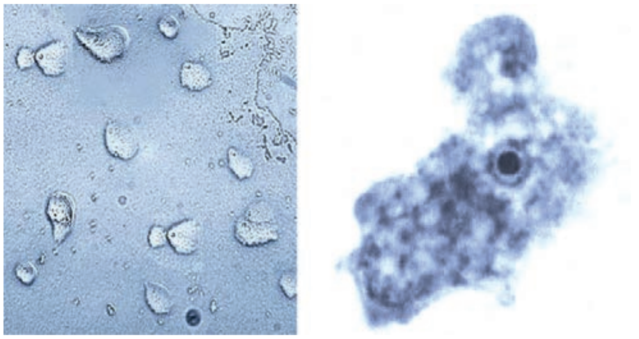


The nonnutrient agar plates are made using Page's saline. Before use, the plates should be removed from the refrigerator and placed at 37°C for 30 min. Add about 0.5 ml of saline to a slant culture of *Escherichia coli*. Gently scrape the surface, and suspend bacteria uniformly. Add 2 or 3 drops of this suspension to the middle of the warmed agar plate. Using a loop, spread the bacteria on the surface of the agar. Depending on the specimen, cerebrospinal fluid (CSF) or other nervous system material should be incubated at 37°C; other tissues should be incubated at 30°C. After the fluid/specimen has been absorbed, seal the plates with a 5- to 6-in. strip of Parafilm; incubate the plate inverted in room air.

Using the low-power objective (10×), examine the plates for amebic cysts or trophozoites every day for 10 days. Thin, linear tracks, which represent areas where amebae have ingested the bacteria, might also be seen.

Current therapy for *Acanthamoeba* infection includes amphotericin B and sulfadiazine or sulfisoxazole or pentamidine isethionate. Treatment is somewhat problematic, and amphotericin B may be ineffective. Skin ulcers have been treated by combination therapy involving systemic intravenous pentamidine isethionate for 1 month and oral itraconazole for 8 months. The skin ulcers can also be cleaned twice daily with chlorhexidine gluconate solution and treated by topical application of 2% ketoconazole cream.

Key Points for Laboratory Identification

1. As with *Naegleria fowleri*, beware of a false-positive Gram stain (the leukocyte count in CSF may be elevated).
2. Examine clinical material on a slide with a coverslip; do not use a counting chamber (organisms in CSF look like leukocytes when placed in a counting chamber).
3. Nonnutrient agar with Page's saline and an *E. coli* overlay can be tried for culture recovery.
4. Various other media containing different agar bases, some of which contain horse, sheep, or rabbit blood, are also available.
5. Tissue stains are also effective, and the use of calcofluor white to visualize the double-walled cyst is recommended.
6. Refer to chapter 8 for specific methods and recommendations.

Case 3

A 6-year-old girl was admitted to the hospital in a coma. The patient was normally a healthy child with no significant medical history. Several days prior to admission, the family had been vacationing at a lake in the southern United States. Everyone had been swimming and boating during the vacation. Several days before admission, the patient had complained of some vague upper respiratory distress and a sore throat. On the day prior to admission, her mother indicated that she had complained of a stiff neck and headache; she also had a fever. On the day of admission, she became very confused and lapsed into a coma.

On physical examination, her neck was stiff and her pupils reacted sluggishly; her temperature was 104°F. The CSF was hemorrhagic and contained many neutrophils. Although there was some debris on the smear, the Gram stain was read as negative. The images in Fig. 12.4 were seen from culture and stained preparations (4–6).

Questions

1. What laboratory procedure should be performed immediately and why?
2. If the procedure was positive, what would you expect to see?
3. Treatment should be started immediately with what and why?
4. What is there about the history that should alert everyone to this disease possibility?

Correct Identification

The CSF should be examined as a wet mount (not in a counting chamber), looking for motile amebic trophozoites. The specimen can be centrifuged or examined as unspun fluid; it should not be refrigerated prior to examination. Amebic trophozoites can mimic neutrophils in a counting chamber, but characteristic motility can be seen on a slide with a coverslip. In a positive wet mount, motile trophozoites of free-living amebae, *Naegleria fowleri*, would be seen. The pseudopodia are rounded and nonspiky, and motility is visible unless the organisms are cold or dead. The only drug that appears to be effective is amphotericin B. Very few patients have survived this infection; two patients responded to amphotericin B and did survive. Rare cases have also responded to miconazole and, in one case, rifampin.

CNS infections caused by free-living amebae have been recognized only since the mid-1960s, and our understanding of this disease process is still incomplete. One type of meningoencephalitis (PAM) is a fulminant and rapidly fatal disease that mainly affects children and young adults. The disease closely resembles bacterial meningitis but is caused by *N. fowleri*, an organism found in moist soil and freshwater habitats.

There are trophozoite and cyst stages in the life cycle; the stage depends primarily on environmental conditions. Trophozoites can be found in water or moist soil and can be maintained in tissue culture or other artificial media. The trophozoites can occur in two forms, ameboid and flagellate. Motility can be observed in hanging-drop preparations from cultures of CSF; the ameboid form (the only form recognized in humans) is elongate with a broad anterior end and tapered posterior end. The size ranges from 7 to 20 µm. The diameter of the rounded forms is usually 15 µm. There is a large, central karyosome and no peripheral nuclear chromatin. The cytoplasm is somewhat granular and contains vacuoles. The ameboid-form organisms change to the flagellate form when they are transferred from culture or teased from tissue into water and maintained at a temperature of 27 to 37°C. The change may occur within a few hours or may take as long as 20 h. The flagellate form is pear shaped, with two flagella at the broad end. Motility is typical, with either spinning or jerky movements. These flagellate forms do not divide, but when the flagella are lost, the ameboid forms resume reproduction. Cysts from nature and from agar cultures look the same and have a single nucleus almost identical to that seen in the trophozoite. The shape is generally round, measuring from 7 to 10 µm, and there is a thick double wall.

PAM caused by *N. fowleri* is an acute, suppurative infection of the brain and meninges. In humans, with extremely rare exceptions, the disease is rapidly fatal. The period between contact with the organism and onset of clinical symptoms such as fever, headache, and rhinitis may vary from 2 to 3 days to as long as 7 to 15 days. The amebae may enter the nasal cavity by inhalation or aspiration of water, dust, or aerosols containing the trophozoites or cysts. The organisms then penetrate the nasal mucosa, probably through phagocytosis of the olfactory epithelium cells, and migrate via the olfactory nerves to the brain. Early symptoms include vague upper respiratory distress, headache, lethargy, and occasionally olfactory problems. The acute phase includes sore throat, stuffy blocked or discharging nose, and severe headache. Progressive symptoms include pyrexia, vomiting, and stiffness of the neck. Mental confusion and coma usually occur approximately 3 to 5 days prior to death. The cause of death is usually cardiorespiratory arrest and pulmonary edema. PAM can resemble acute purulent bacterial meningitis, and the conditions may be difficult to differentiate, particularly in the early stages. Unfortunately, if the CSF Gram stain is interpreted incorrectly, with the identification of bacteria representing a false-positive result, the antibacterial therapy has no impact on the amebae and the patient will usually die within several days. Extensive tissue damage occurs along the path of amebic invasion; the nasopharyngeal mucosa shows ulceration, and the olfactory nerves are inflamed and necrotic. Hemorrhagic necrosis is concentrated in the region of the olfactory bulbs and the base of the brain. Organisms can be found in the meninges, perivascular spaces, and sanguinopurulent exudates.

Clinical and laboratory data usually cannot be used to differentiate pyogenic meningitis from PAM, so the diagnosis may have to be reached by a process of elimination. A high index of suspicion is often mandatory for early diagnosis. Although most cases are associated with exposure to contaminated water through swimming or bathing, this is not always the case. The rapidly fatal course of 4 to 6 days after the beginning of symptoms, with an incubation period of 1 day to 2 weeks, requires early diagnosis and immediate chemotherapy if the patient is to survive. Analysis of the CSF shows decreased glucose and increased protein concentrations. Leukocytes may range from several hundred to >20,000 cells/mm^3. Gram stains and bacterial cultures of CSF are negative.

A definite diagnosis could be made by demonstration of the amebae in the CSF or in biopsy specimens. Either CSF or sedimented CSF should be placed on a slide, under a coverslip, and observed for motile trophozoites; smears can also be stained with Wright's or Giemsa stain. CSF, exudate, or tissue fragments can be examined by light microscopy or phase-contrast microscopy. Care must be taken not to mistake leukocytes for actual organisms or vice versa. It is very easy to confuse leukocytes and amebae, particularly when one is examining CSF by using a counting chamber, hence the recommendation to use just a regular slide and coverslip. Motility may vary, so the main differential characteristic is the spherical nucleus with a large karyosome.

Specimens should never be refrigerated prior to examination. When centrifuging the CSF, low speeds (250 × *g*) should be used so that the trophozoites are not damaged; however, centrifugation at 500 × *g* is not detrimental to the morphology. Although bright-field microscopy with reduced light is acceptable, phase microscopy, if available, is recommended. Use of smears stained with Giemsa or Wright's stain or a Giemsa-Wright's stain combination can also be helpful. If *Naegleria* is the causative agent, trophozoites only are normally seen. If the infecting organism is *Acanthamoeba*, cysts may also be seen in specimens from the CNS. Unfortunately, most cases of PAM are diagnosed at autopsy; confirmation of these tissue findings must include culture and/or special staining using monoclonal reagents in indirect fluorescent-antibody procedures. Organisms can also be cultured on nonnutrient agar plated with *E. coli*.

Over 175 presumptive or proven cases of PAM have been reported in the literature, including cases from the United States (86 cases as of January 1998), Ireland, England, Belgium, Czechoslovakia, Australia, New Zealand, Brazil, and Zambia. Clinical patient histories indicate exposure to the organism via freshwater lakes or swimming pools shortly before onset; patients had been previously healthy with no specific underlying problems. Pathogenic *Naegleria* organisms have also been isolated from nasal passages of individuals with no history of water exposure, suggesting the possibility of airborne exposure.

The first isolations of environmental strains of pathogenic *N. fowleri* were reported from water and soil in Australia and from sewage sludge samples in India. Detection of *N. fowleri* in heated discharge water has been reported in Belgium and Poland. Since then, there have been additional reports describing isolations of virulent or avirulent strains of *N. fowleri* from the environment. Studies in Belgium have clearly indicated that *N. fowleri* strains are present in artificially heated waters (power plant warm discharge), and studies in the United States have indicated that virulent strains are also found in lakes at geographic latitudes with water temperatures of 14 to 35°C that are totally isolated from any source of thermal discharge. These data suggest that the presence of pathogenic or potentially pathogenic amebic strains may depend on both climate and modification of the natural environment. The ability of the cysts to survive under various environmental conditions has been investigated by several workers. These findings suggest that *N. fowleri* cysts produced in the warm summer months may survive the winter and are capable of growth during the following summer.

General preventive measures include public awareness of potential hazards of contaminated water. It has been recommended that warm discharge water not be used for sports and recreational purposes, particularly since DNA restriction fragment profiles of environmental strains and human isolates were homogeneous.

Only 3 patients of more than 175 with presumptive or parasitologically proven cases of PAM had survived this infection outside of the United States by the 1970s. Since 1962, all 117 cases of PAM confirmed in the United States have been limited to exposure in southern-tier states (Arizona, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, Missouri, Nevada, New Mexico, North Carolina, Oklahoma, South Carolina, Texas, and Virginia). The first confirmed case of PAM acquired in Minnesota has been reported, the northernmost state in which this infection has been documented (7, 8). One case within California was documented in which the patient was successfully treated with amphotericin B, miconazole, and rifampin. There was a synergistic effect with miconazole and amphotericin B *in vitro*; however, rifampin was ineffective. The patient received intrathecal and intravenous injections of the drug. Dexamethasone and phenytoin were also given to this patient to decrease intracranial pressure and seizure, respectively. *Naegleria* infections have also been treated successfully with amphotericin B, rifampin, and chloramphenicol; amphotericin B, oral rifampin, and oral ketoconazole; and amphotericin B alone. Miltefosine generally is well tolerated, with gastrointestinal symptoms the most commonly reported adverse effects. Miltefosine is a drug used to treat leishmaniasis and also has shown *in vitro* activity against free-living amebae, but as an investigational drug, it has not been readily available in the United States. With CDC assistance, however, miltefosine has been administered since 2009 for these infections as single-patient emergency use with permission from the Food and Drug Administration. Clinicians who suspect they have a patient with free-living amebic infection who could benefit from treatment with miltefosine should contact CDC to consult with an expert. For diagnostic assistance, specimen collection guidance, specimen shipping instructions, and treatment recommendations, clinicians should contact the CDC Emergency Operations Center at 770-488-7100 (7).

Key Points for Laboratory Identification

1. Never refrigerate the specimen(s) prior to examination.
2. Beware of the false-positive Gram stain, especially since PAM commonly mimics pyogenic meningitis with the CSF containing increased numbers of leukocytes.
3. *Naegleria* trophozoites mimic leukocytes if put in a counting chamber. Motility is more likely to be seen if a drop of CSF is placed directly on a slide and a coverglass is added.
4. Organisms can be cultured on nonnutrient agar plated with *E. coli*.

Case 4

An immunocompetent 5-year-old girl was diagnosed with diarrhea off and on for about 10 days, and two stool specimens were submitted to the laboratory for examination. After examination of the concentration sediment from both specimens, nothing was seen and the results were reported as "No parasites seen" (Fig. 12.5, left). No permanent stained smears were prepared or examined. The child continued to be symptomatic with vague gastrointestinal problems and diarrhea. Another stool specimen was submitted, and both concentration sediment and permanent stained smears were examined. The parasite identified is seen in Fig. 12.5 (right). This protozoan trophozoite measures approximately 13 to 16 μm long (1–6).

Questions

1. Would you agree or disagree with the original diagnostic approach used for the first two stool samples?
2. If one considers all the intestinal protozoa, which organisms might have been missed without the permanent stained smear examination?
3. Identification of which organism requires the permanent stained smear? Why is this true?
4. What references might be helpful in reviewing guidelines for a complete O&P examination?

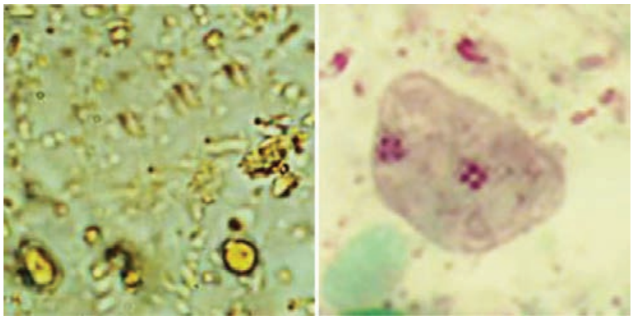


Figure 12.5 Case 4. (Left) Stool fecal concentration sediment examined as a wet mount (appears to be negative); (right) protozoan trophozoite containing two nuclei, which are fragmented into several nuclear granules.
doi:10.1128/9781555819002.ch12.f5

Correct Identification

The organism image presented is a trophozoite of the flagellate *Dientamoeba fragilis*; thus, the physician's report ("No parasites seen") was incorrect. The first report was based on examination of the concentration sediment wet preparation (Fig. 12.5, left), in which no organisms were seen.

D. fragilis was first seen in 1909 but not described until 1918. On the basis of electron microscopy studies, it has been reclassified as an ameba-flagellate rather than an ameba and is closely related to *Histomonas* and *Trichomonas* spp. It has a cosmopolitan distribution, and past surveys showed incidence rates of 1.4 to 19%. Much higher incidence rates have been reported for mental institution inmates, missionaries, and Native Americans in Arizona. *D. fragilis* tends to be common in some pediatric populations, and in some studies the incidence figures are higher for patients younger than 20 years. Trophozoites can contain one or two nuclei, and a cyst form has recently been identified for this organism (9).

Although its pathogenic status is still not well defined, *D. fragilis* has been associated with a wide range of symptoms and is considered a definite emerging pathogen. Case reports of children infected with *D. fragilis* reveal a number of symptoms including intermittent diarrhea, abdominal pain, nausea, anorexia, malaise, fatigue, poor weight gain, and unexplained eosinophilia. The most common symptoms in patients infected with this parasite appear to be intermittent diarrhea and fatigue. In some patients, both the organism and the symptoms persist or reappear until appropriate treatment is initiated.

Diagnosis of *D. fragilis* infections depends on proper collection and processing techniques (a minimum of three fecal specimens). Although survival time for this parasite has been reported as 24 to 48 h, the survival time in terms of morphology is limited, and stool specimens must be examined immediately or preserved in a suitable fixative soon after defecation. It is particularly important that permanent stained smears of stool material be examined with an oil immersion lens (1,000×). These organisms have been recovered in formed stool; therefore, a permanent stained smear must be prepared for every stool submitted for a parasite examination.

If preserved specimens are submitted to the laboratory, it may be more cost-effective to delete the direct smear and begin the stool examination with the concentration procedure, particularly since motile protozoa are not viable because of the prior addition of preservative. Even if parasites are seen on a direct mount of preserved stool, they would almost certainly be seen on the concentration examination as well as on the permanent stained smear (protozoa in particular). With few exceptions, intestinal protozoa should never be identified on the basis of a wet mount alone; permanent stained smears should be examined to confirm the specific identification of suspected organisms.

Every stool submitted for an O&P examination must be examined using the concentration and permanent stained smear procedures. This guideline is consistent with the College of American Pathologists laboratory inspection checklist.

Key Points for Laboratory Diagnosis

1. O&P examination (fresh stool specimen, liquid and/or very soft): direct wet smear, concentration, permanent stained smear.
2. O&P examination (fresh stool specimen, formed stool): concentration, permanent stained smear.
3. O&P examination (preserved stool specimen): concentration, permanent stained smear.

All intestinal protozoan infections can be missed if the concentration is the only test to be performed. The permanent stained smear is much more sensitive than the concentration alone. To date, there are no commercial immunoassay products available for the confirmation of infection with *D. fragilis*, although they are under development.

Case 5

A 3-year-old boy was first seen in the clinic because of failure to thrive and vague symptoms such as poor appetite and failure to gain weight. There was one other child in the family, a 6-year-old brother who was in good health. The patient was apparently fine for the first couple of years. He began to have diarrhea with light-colored stools; however, this condition was not always present. Although stool examinations were performed, it was unclear exactly what diagnostic methods were used and the test results were reported as negative. The child was placed on a high-protein, high-calorie diet with vitamins and supplements. However, he showed very little improvement over a 4-month period. Based on these and other findings (barium exam showed "large dilated loops of hypotonic bowel"), the child was admitted to the hospital with a diagnosis of celiac disease.

Complete stool examinations were performed (O&P examination: direct wet mount, concentration, and permanent stained slide). The image in Fig. 12.6 is a wet preparation of stool; the organism was seen using the high dry objective of the microscope. Motility was seen, but it was very limited and the organism identification could not be confirmed without completion of the O&P examination (1–6). The images in Fig. 12.6 were photographed at a high magnification.

Questions

1. What parasites should be considered based on the patient's history?
2. What factors might be responsible for the "negative" laboratory reports?
3. After seeing the organisms in the wet mounts, could these parasites be responsible for the patient's symptoms?
4. What other diagnostic tests might be available? Would they be more or less sensitive than the O&P examination? Please explain.

Correct Identification

Fig. 12.6 shows a *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) trophozoite (left) and a *G. lamblia* (*G. duodenalis*,

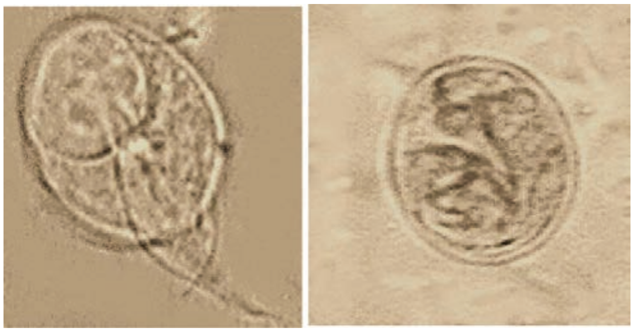


Figure 12.6 Case 5. (Left) Protozoan trophozoite seen in a wet mount; (right) protozoan cyst seen in a wet mount (note how round, distorted, and three-dimensional the cyst appears. doi:10.1128/9781555819002.ch12.f6

G. intestinalis) cyst (right) in a wet preparation. The trophozoite measured approximately 8 by 12 µm, and there appeared to be minimal motility related to the flagella; the cyst measured approximately 10 µm and appeared to be somewhat round. Although this organism was seen in the wet mount, additional morphology could be seen from the concentration and permanent stained slide examinations. While the presumptive identification could be made from the concentration wet mount, confirmation of the diagnosis usually depends on examination of the permanent stained smear (1–6).

The incubation time for giardiasis is approximately 12 to 20 days. Because the acute stage usually lasts only a few days, giardiasis may not be recognized as the infection but may mimic acute viral enteritis, bacillary dysentery, bacterial or other food poisonings, acute intestinal amebiasis, or “traveler’s diarrhea” (caused by toxigenic *E. coli*). However, the type of diarrhea plus the lack of blood, mucus, and cellular exudate is consistent with giardiasis. Although the organisms in the crypts of the duodenal mucosa may reach very high densities, they may not cause any pathology. The organisms feed on the mucous secretions and do not penetrate the mucosa. For some reason, in symptomatic cases there may be irritation of the mucosal lining, increased mucus secretion, and dehydration.

The acute phase is often followed by a subacute or chronic phase. Symptoms in these patients include recurrent, brief episodes of loose, foul-smelling stools; there may be increased distention and foul flatus. Between the episodes of passing mushy stools, the patient may have normal stools or may be constipated. Abdominal discomfort continues to include marked distention and belching with a rotten-egg taste. Chronic disease must be differentiated from amebiasis and other intestinal parasites such as *Dientamoeba fragilis*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora belli*, and *Strongyloides stercoralis* and from inflammatory bowel disease and irritable colon. Based on symptoms such as upper intestinal discomfort, heartburn, and belching, giardiasis must also be differentiated from duodenal ulcer, hiatal hernia, and gallbladder and pancreatic disease. Because giardiasis may not cause any symptoms at all, demonstration of the organism in symptomatic patients may not rule out other possibilities such as peptic ulcer, celiac disease, strongyloidiasis, and possibly carcinoma.

Giardiasis is one of the more common causes of traveler’s diarrhea and has been recorded from all parts of the world. It has also been speculated that visitors to areas where *Giardia* is endemic are more likely to present with symptoms than are individuals who live in the area; this difference is most probably due to the development of immunity from prior, and possible continued, exposure of residents to the organism.

Both the trophozoite and the cyst are included in the life cycle of *G. lamblia* (*G. duodenalis*, *G. intestinalis*). Trophozoites divide by means of longitudinal binary fission, thus producing two daughter trophozoites. The most common location of the organisms is in the crypts within the duodenum. The trophozoites are the intestinal dwelling stage and attach to the epithelium of the host villi by means of the ventral disk. The attachment is substantial and results in disk “impression prints” when the organism detaches from the surface of the epithelium. Trophozoites may remain attached or may detach from the mucosal surface. The epithelial surface sloughs off the tip of the villus every 72 h, and apparently the trophozoites detach at that time. For reasons which are not totally known, cyst formation takes place as the organisms move down through the colon. The trophozoites retract the flagella into the axonemes, the cytoplasm becomes condensed, and the cyst wall is secreted. As the cyst matures, the internal structures are doubled, so that when excystation occurs, the cytoplasm divides, thus producing two trophozoites. Excystation would normally occur in the duodenum.

Routine stool examinations are normally recommended for the recovery and identification of intestinal protozoa. However, in the case of *G. lamblia* (*G. duodenalis*, *G. intestinalis*) because the organisms are attached so securely to the mucosa by means of the sucking disk, a series of even five or six stool specimens may be examined without recovering the organisms. The organisms also tend to be passed in the stool on a cyclical basis. The Entero-Test capsule can be helpful in recovering the organisms, as can the duodenal aspirate. Although cysts can often be identified on the wet stool preparation, many infections may be missed without the examination of a permanent stained smear. If material from the string test (Entero-Test) or mucus from a duodenal aspirate is submitted, it should be examined as a wet preparation for motility; however, motility may be represented by nothing more than a slight flutter of the flagella because the organism is caught up in the mucus. After diagnosis, the rest of the positive material can be preserved as a permanent stain.

Procedures using immunoassays (enzyme immunoassay, fluorescent-antibody assay, cartridge formats) have also been developed to detect *Giardia* antigen in feces. The immunoassays are more sensitive than the O&P examination. Many of these newer methods are being used to diagnose patients suspected of having giardiasis or those who may be involved in an outbreak situation, particularly if the patient history is suggestive. However, due to shedding variability, if the first stool immunoassay is negative, a second stool should be tested (this is not required for *Cryptosporidium*; one stool is sufficient).

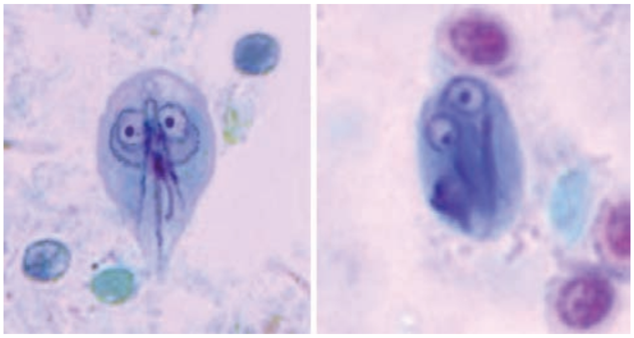
Transmission is by ingestion of viable cysts. Although contaminated food or drink may be the source, transmission may occur via intimate contact with an infected individual. This organism tends to be found more frequently in children or in groups that live in close quarters. Often there are outbreaks due to poor sanitation facilities or breakdowns as evidenced by infections in travelers and campers. There may also be an increase in the prevalence of giardiasis in the male homosexual population, probably due to anal and/or oral sexual practices. Because of the potential for a wild-animal reservoir and possibly other domestic-animal reservoir hosts, measures in addition to personal hygiene and improved sanitary measures have to be considered. Iodine has been recommended as an effective disinfectant for drinking water, but it must be used according to directions. Filtration systems have also been recommended, although they have certain drawbacks such as clogging.

Most experts agree that the single most effective practice that prevents the spread of infection in the child care setting is good handwashing by the children, staff, and visitors. Rubbing hands together under running water is the most important part of washing away infectious organisms. Premoistened towelettes or wipes and waterless hand cleaners should not be used as a substitute for washing hands with soap and running water. These guidelines are not limited to giardiasis but include all potentially infectious organisms.

Key Points for Laboratory Diagnosis

1. Even if a series of three stool specimens is submitted and examined correctly, the organisms may not be recovered and identified. The organisms are shed on a sporadic basis.
2. Motility on wet preparations may be difficult to see because the organisms may be caught up in mucus.
3. Any examination for parasites in stool specimens must include the use of a permanent stained smear (even on formed stool) (Fig. 12.7).
4. Duodenal drainage and/or the use of the Entero-Test capsule may be very helpful in organism recovery. However, this technique does not take the place of the O&P examination. Fecal immunoassays for antigen detection are now widely used and include the enzyme immunoassay, fluorescent-antibody assay, and cartridge formats. Based on the patient's history, a fecal immunoassay performed on two separate stool specimens is more appropriate and sensitive than the O&P. However, it is important to remember that patients who are carriers and do not present with acute symptoms may have insufficient numbers of organisms for a positive fecal immunoassay result, even though the immunoassays are more sensitive than the O&P.

Figure 12.7 Case 5. (Left) *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) trophozoite seen in a permanent stained smear; (right) *Giardia* cyst seen in a permanent stained smear (note how much more detail is seen compared to the wet mounts in Fig. 12.6). doi:10.1128/9781555819002.ch12.f7



If giardiasis is diagnosed, the patient should be treated. In the majority of cases, metronidazole is the drug of choice, but it is still considered investigational. Metronidazole is not recommended for pregnant women; paromomycin may be used to treat giardiasis in pregnancy, although it is not absorbed and not highly effective. Tinidazole has also been used and has proven to be more effective than metronidazole as a single dose. Furazolidone is another option but has been reported to be mutagenic and carcinogenic.

In the absence of a parasitologic diagnosis, the treatment of suspected giardiasis is a common question with no clear-cut answer. The approach depends on the alternatives and the degree of suspicion of giardiasis, both of which vary among patients and physicians. However, it is not recommended that treatment be given without a good parasitologic workup, particularly since relief of symptoms does not allow a retrospective diagnosis of giardiasis; the most commonly used drug, metronidazole, targets other organisms besides *G. lamblia*. Some patients have been successfully treated with the combination of paromomycin and metronidazole

Should asymptomatic patients be treated? Generally, it is recommended that all cases of proven giardiasis be treated because the infection may cause subclinical malabsorption, symptoms are often periodic and may appear later, and a carrier is a potential source of infection for others. Certainly in areas of the world where infection rates, as well as the prospect of reinfection, are extremely high, the benefit-per-cost ratio would also have to be examined.

Case 6

A 5-year-old female was first seen in the clinic because of failure to thrive and vague symptoms including poor appetite and failure to gain weight. The patient was apparently fine until about 2 months prior to becoming symptomatic.

She began to have diarrhea, although this condition was not always present. She exhibited a lack of appetite and general malaise, while at times she appeared to be normal with no particular health problems. Although stool examinations were performed, the initial examinations were reported as negative. Information regarding the original number and types of stool examinations performed was unavailable; the family had just moved from another state approximately 3 weeks before.

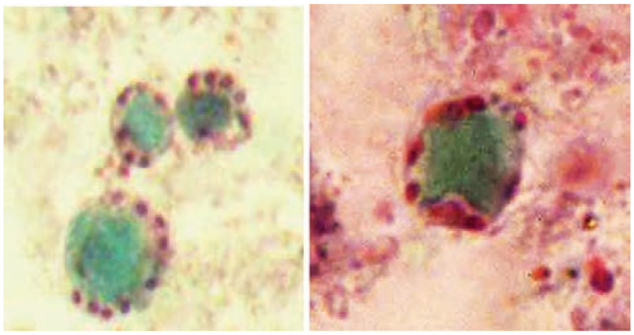
Additional parasitology examinations were performed on stool specimens that had been collected in the single-vial system (Universal Fixative—TOTAL-FIX) (O&P examination: concentration, permanent stained slide). Note that the direct wet mount was not performed since the specimen was submitted in preservative; this is the correct approach. The direct wet mount is not performed unless fresh stool is submitted, and the specimen is liquid or very soft.

Laboratory results revealed the images in Fig. 12.8 on the second and third stool examinations from the trichrome-stained smear; the first specimen was reported as "No parasites seen." The image is from a trichrome-stained permanent stained smear; the organism was seen using the 100× oil immersion objective of the microscope. Although a specific organism was not suspected from the history, organism identification could not be confirmed without examination of the permanent stained slide, and this was the only protozoan type seen in the entire examination.

Questions

1. From this image seen on the permanent stained smear, can you identify the organism to the genus and species levels?
2. What factors might be responsible for the "negative" laboratory reports?
3. How should this organism be reported? Could this organism be responsible for the patient's symptoms?
4. What factors have led to the pathogenicity controversy surrounding the reporting of this organism?

Figure 12.8 Case 6. Microscopic images (oil immersion 100× objective); permanent stained smear.
doi:10.1128/9781555819002.ch12.f8



Correct Identification

This image contains several *Blastocystis* spp. central body forms; these organisms measured approximately 10 to 25 µm. The organisms were reported as "moderate *Blastocystis* spp. (*Blastocystis hominis*)" seen.

Blastocystis spp. are common intestinal parasites, with a prevalence in developing countries of up to 50%. Infection has been reported as asymptomatic, acute symptomatic, and chronic symptomatic. *Blastocystis* spp. are among the most commonly observed intestinal parasites in routine clinical parasitology stool examinations. *Blastocystis* in humans consists of at least nine genetic subtypes, which may be associated with differences in pathogenicity and symptomatology. This wide range of responses to infection could be related to the genetic diversity of morphologically indistinguishable specimens obtained from infected hosts. The former name *Blastocystis hominis* is now reported as *Blastocystis* spp. because of its genetic diversity. *Blastocystis* is recognized as a complex of subtypes that have not been fully characterized as independent species. Molecular studies have successfully classified subtypes or subgroups among *Blastocystis* strains and also revealed the presence of zoonotic strains. In light of the genetic similarities and differences observed among human and nonhuman *Blastocystis* isolates, it is reasonable to speculate that the transmission of organisms occurs either by human-to-human or animal-to-human routes. Also, results support the hypothesis that asymptomatic and symptomatic isolates may represent different strains, species, or subtypes with varying pathogenic potential. However, all the organisms isolated from patients have similar morphologies; thus the appearance does not indicate pathogen versus nonpathogen.

Based on small-subunit rDNA analysis, this genus comprises at least 17 lineages (arguably species), the so-called subtypes (STs) ST1 to ST4 account for probably more than 90% of human carriage, while the remaining human carriers are colonized by isolates belonging to ST5 to ST9. All of these STs, apart from ST9, have also been found in various nonhuman hosts. Since 2009, no fewer than eight novel STs (ST10 to ST17) have been detected in nonhuman hosts, primarily by amplification of *Blastocystis* DNA directly from fecal DNA template (10, 11).

Though associated with self-limiting infections, treatment is required in many patients who continue to be symptomatic. However, different subsets of *Blastocystis* exhibit resistance to metronidazole and are associated with variable degrees of symptoms. Therapy should be limited to patients with persistent symptoms subsequent to a complete work up for alternative etiologies.

Blastocystis is a single-celled stramenopile, is capable of pseudopod extension and retraction, reproduces by binary fission or sporulation, and has a membrane-bound central body (previously called a vacuole) that takes up 90% of the cell and functions in sexual and asexual reproduction. Other structures with unknown function are not yet defined. The organism is strictly anaerobic, normally requires bacteria for growth, and is capable of ingesting bacteria and other debris.

The organism is polymorphic with only few of forms such as vacuolar, granular, amoeboid, and the cyst form being commonly known. The classic form that is usually seen in the human stool specimen varies tremendously in size, from 6 to 40 µm, and is characterized by a large central body, which may be involved with carbohydrate and lipid storage (visually like a large vacuole). The more amebic form is occasionally seen in diarrheal fluid but may be extremely difficult to recognize. Generally, *Blastocystis* will be identified on the basis of the more typical round form with the central body ("central body form").

Both thin- and thick-walled cysts have been confirmed. It is suggested that the thin-walled cysts are autoinfectious, leading to multiplication of the organism in the intestinal tract. The thick-walled cysts are probably responsible for external transmission via the fecal oral route. This type of life cycle might explain the high percentage of positive carriers in many studies, showing that the percentage of patients with *Blastocystis* is often much higher than those who are infected with other intestinal protozoa. However, autoinfection has not been confirmed.

When *Blastocystis* is present in the absence of other pathogenic parasites, bacteria, or viruses, it may be the cause of diarrhea, cramps, nausea, fever, vomiting, and abdominal pain and may require therapy. In one recent study of patients with irritable bowel syndrome, there was a set of patients in whom the presence of this organism did not appear to be incidental. Possible relationships between *Blastocystis* and intestinal obstruction and perhaps even infective arthritis have been suggested. In patients with other underlying conditions, the symptoms may be more pronounced. The incidence of this organism appears to be higher than suspected in stools submitted for parasite examination worldwide. In symptomatic patients in whom no other etiologic agent has been identified, *Blastocystis* should certainly be considered the possible pathogen.

Routine stool examinations are very effective in recovering and identifying *Blastocystis*, although the permanent stained smear is the procedure of choice since the examination of wet preparations may not easily reveal the organism (Fig. 12.8). If the fresh stool is rinsed in water before fixation (for the concentration method), these organisms, other than the cysts, will be destroyed, thus possibly yielding a false-negative report. The organisms should be quantitated on the report form, i.e., as rare, few, moderate, or many. It is also important to remember that other possible pathogens should be adequately ruled out before a patient is treated for *Blastocystis*.

Key Points for Laboratory Diagnosis

1. Do not prerinse the specimen with water; organisms will rupture, thus leading to a false-negative result.
2. *Blastocystis* is the most common intestinal protozoan seen in stool worldwide (even more common than *Giardia lamblia* [*G. duodenalis*, *G. intestinalis*] or *Dientamoeba fragilis*); learn to recognize and report (quantitate) these organisms.
3. There may be tremendous variation in size, even in a single stool specimen.
4. Although other stages may be present, the central body form is the most common and easiest to recognize.

Remember, there is still controversy regarding quantitation of these organisms on the laboratory report form. Although there was thought to be correlation between numbers of organisms present and patient symptoms, some physicians have seen symptomatic patients where even small numbers of *Blastocystis* are present and there appear to be no other causative agents present in the stool specimen(s). For this reason, quantitation of the organisms is still recommended. However, it is important to communicate with your physician clients so that they know how you are reporting and/or quantitating this organism. The approach must be determined by each laboratory after discussions with physician clients.

Case 7

A 38-year-old male, originally from Los Angeles, was seen in the Tropical Medicine Clinic with a 3-week history of diarrhea and general malaise. This patient was a buyer of raw materials for his company, and he traveled throughout the world visiting suppliers on a regular basis. These trips included Europe, Latin America, Asia, Africa, and throughout North America. He often took side trips on his travels and tended to stay in both expensive and relatively inexpensive accommodations. He also liked to eat many of the local dishes on his travels. Apparently, he had no underlying health problems. On presentation to the clinic, he was complaining of intermittent diarrhea, a weight loss of approximately 10 pounds, and general malaise.

Routine stool examinations for parasites (three O&P examinations) revealed the images shown in Fig. 12.9. The images were photographed from the permanent trichrome-stained smear using the 100× objective for a total magnification of ×1000 (1–6).

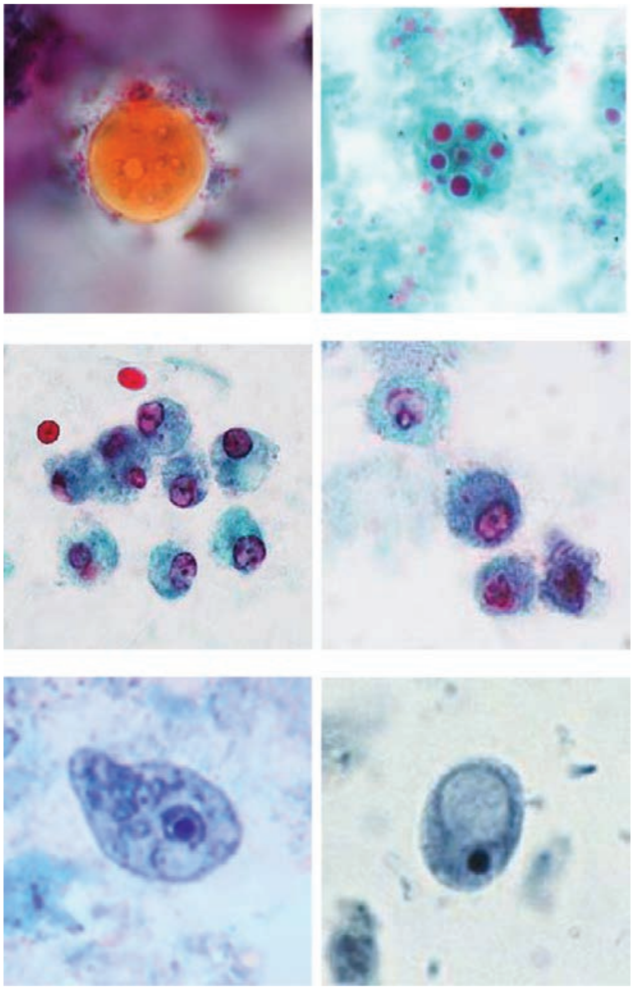
Questions

1. What organisms are suggested from the images shown in Fig. 12.9?
2. What groups of parasites might be involved, and are these typical based on his travel history?
3. What else do these objects suggest?
4. How likely is it that the patient has a mixed infection (based on the structures seen? Why or why not?
5. What other tests might be requested before all intestinal parasites could be eliminated as possibilities?

Correct Identification

The orange structure (Fig. 12.9, upper row, left) resembles an *Entamoeba coli* cyst. However, the "nuclei" are not the same size and the morphology is not uniform enough to be a protozoan cyst. The structure in the upper row, right, resembles a protozoan cyst. The images in the second row mimic protozoan trophozoites and cysts. The images also suggest various artifacts and/or human cells that might be present in human fecal specimens. Based on the images you have seen, it is unlikely that the patient has a mixed infection with multiple parasites. All of the structures in the first row are artifacts, while human cells are seen in the second row. However, with the presence of artifacts and human cells in the specimen, examination of the stained fecal smears should be very thorough in order to "rule out" the possibility of true parasites. Further examination on the permanent stained smear revealed images seen in the third row, actual intestinal protozoa. Because routine stool examinations do not "rule out" all the coccidia and/or the microsporidia, additional testing should be performed. Those tests would include immunoassays for *Cryptosporidium*/*Giardia* or *Cryptosporidium*, and special staining for *Cyclospora cayetanensis* (modified acid-fast stains) and the microsporidia (modified trichrome stains).

Figure 12.9 Case 7. Microscopic images (oil immersion 100× objective); permanent stained smear.
doi:10.1128/9781555819002.ch12.f9



Although the orange structure is the same size as an *E. coli* cyst, the "nuclei" are quite different sizes, a finding that makes the structure unlikely to be a protozoan cyst. Also, *E. coli* cysts do not tend to stain well on the permanent stained smear (fixative does not penetrate the cyst wall very well, even using mercury-based fixatives), but their color tends to be more blue or purple (normal cyst); if the cyst is very shrunk, it may even appear pink to red to purple in color (Wheatley's trichrome stain), rather than being this color orange.

Although the cells (Fig. 12.9, second row) tend to resemble protozoan trophozoites and cysts, notice that the cell nuclei tend to take up a very large part of the cell; this large ratio of nuclear material to cytoplasm (about 1:2 to 1:4) is much more typical of white blood cells (WBCs), not intestinal protozoa. The cell in the center (second row, right image) is probably a macrophage with a single nucleus; these can tend to mimic *Entamoeba histolytica*/*E. dispar* trophozoites. However, actual protozoan trophozoites tend to have a ratio of nucleus to cytoplasm of about 1:8 to 1:12. The other cells are polymorphonuclear leukocytes (PMNs); they can mimic cysts of *Entamoeba histolytica*/*E. dispar*. The lobed nucleus of the PMN tends to look normal (as it would on a routine hematology smear) if the cells are fresh in the stool; however, if the PMNs have been in the gut for a while, the nuclei tend to come apart and the resulting structure can mimic a protozoan cyst.

The organisms in the bottom row are *Iodamoeba bütschlii*; the image on the left is a trophozoite and that on the right is a cyst. Although this organism is considered nonpathogenic, nonpathogens should ALWAYS be reported. Pathogenic and nonpathogenic protozoa are acquired by the human host the same way, the fecal/oral route, the patient having ingested something contaminated with fecal material containing the parasites. However, without additional testing to rule out the coccidia and microsporidia, this case represents a situation in which the cause of the patient's symptoms may be something other than an infection with pathogenic intestinal parasites.

Key Points for Laboratory Diagnosis

1. Human WBCs can often mimic intestinal protozoan trophozoites and cysts; the ratio of nuclear material to cytoplasm is much greater in human cells (1/2 to 1/4), while the ratio in protozoa tends to be much less (1/6 to 1/10).
2. The overall staining appearance using permanent stains will be almost identical between true protozoa and human cells.
3. Both protozoa and human cells may be present in the same stool specimen.
4. Remember that intestinal protozoa tend to be shed sporadically; thus, two (minimum) or three (recommended) specimens are recommended for examination (O&P examination).

Helminth Infections

Case 8

A 28-year-old postdoctoral student presented to his physician with complaints of recurrent abdominal cramps. He had grown up in Italy, come to the United States for graduate studies, and returned home each year for the holidays. Over a period of several years he had complained of recurrent abdominal cramps; however, the causes were thought to be changes in diet, as well as the usual stress associated with graduate school. During the past few months, the symptoms had become worse and he occasionally had diarrhea as well. There was some speculation that he might have an ulcer.

On presentation, the patient appeared in no particular distress, and the examination was unremarkable. The one exception was some slight discomfort on palpation of the abdomen. A gastrointestinal series was normal; however, stool examinations revealed the objects shown in Fig. 12.10. The objects in the upper row measured about 30 by 47 μm and were easily seen in the saline wet preparation examination. Note the suggestion of filaments that lie between the egg shell and the embryo. However, the structures seen in the lower row (permanent trichrome-stained smear) appeared to be somewhat shrunk and distorted.

Questions

1. Which group of parasites includes these objects?
2. Is there anything about the object morphology that suggests the identification?
3. If you know what is causing the infection, do patients usually present with symptoms?
4. What key characteristic of the life cycle plays a role in transmission?

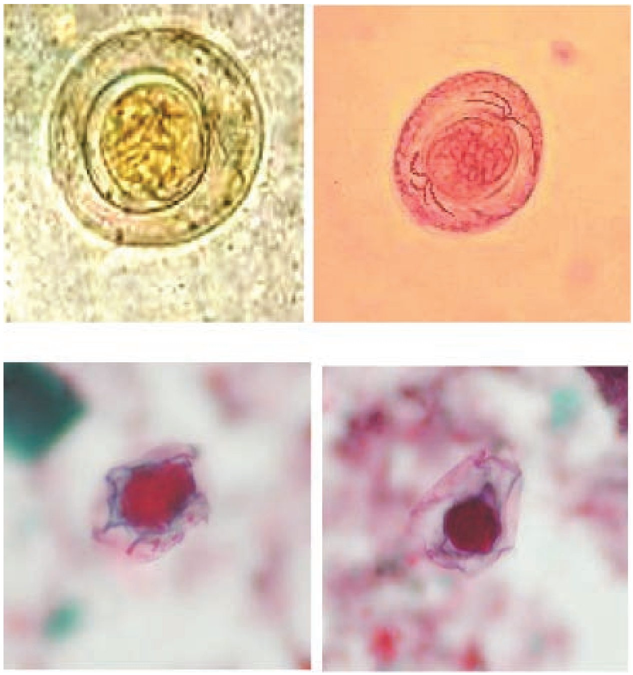


Figure 12.10 Case 8. Helminth eggs. (Upper row) Wet mounts of concentration sediment. Note the polar filaments that lie between the oncosphere and the egg shell. (Bottom row) Permanent stained smear, *Hymenolepis nana* eggs or artifacts. These images may or may not be actual *H. nana* eggs. On permanent stained smears, helminth egg morphology often appears distorted with very dark staining. doi:10.1128/9781555819002.ch12.f10

Correct Identification

These images are *Hymenolepis nana* eggs; these eggs generally measure 30 to 47 μm in diameter. They are round to oval, contain a six-hooked oncosphere (embryo), and have *polar filaments that lie between the egg shell and the developing embryo*.

Since the adult worms or proglottids are rarely seen in the stool, the diagnosis is based on recovery and identification of the characteristic eggs. They are most easily seen and identified from fresh specimens or those preserved in formalin-based fixatives. The thin-shelled eggs also tend to collapse on the permanent stained smear and may be difficult to identify in this type of preparation (1–6). Eggs with the characteristic thin shell, six-hooked oncosphere, and polar filaments are diagnostic. Remember, the eggs are infectious, and unpreserved stool specimens should be handled with caution.

An infection with *H. nana* may cause no symptoms, even with a heavy worm burden. Some patients complain of headache, dizziness, anorexia, abdominal pain, diarrhea, or possibly irritability. Some patients have a low-grade eosinophilia of 5% or more. When young children have heavy infections, they may have loose stools or even diarrhea containing mucus. Persistent, diffuse abdominal pain seems to be the most common symptom.

Infection is usually acquired by the ingestion of *H. nana* eggs, primarily from human stool. The eggs hatch in the stomach or small intestine, and the liberated larvae (oncospheres) penetrate the villi in the upper small intestine. The larvae develop into the cysticercoid stage in the tissue and migrate back into the lumen of the small intestine, where they attach to the mucosa. The adult worms mature within several weeks; they measure up to 40 mm long. Adult worms are rarely seen in the stool. This life cycle involves transition from the egg to the adult worm within the human; no intermediate host is required. Although accidental ingestion of the insect intermediate host can result in development of the adult worms, this mode of infection is probably rare. There is evidence that auto- or hyperinfection occurs when eggs spontaneously hatch in the small intestine and initiate a new life cycle. Although adult worms live for only about a year, some patients carry a large worm burden for many years, probably due to the autoinfection capability within the life cycle.

H. nana is the only human tapeworm in which an intermediate host is not required and transmission is from person to person. Children are usually infected more often than adults. Since infection is from person to person via the eggs, good personal hygiene is an important preventive measure.

Niclosamide has been widely used for years. However, the use of praziquantel in a single oral dose is apparently more effective and kills the cysticercoid as well as the adult worm. The recommended dose for adults and children is 25 mg/kg in a single dose.

Key Points for Laboratory Diagnosis

1. Adult worms or proglottids are rarely seen in the stool.
2. Eggs with the characteristic thin shell, six-hooked oncosphere, and polar filaments are diagnostic.
3. Egg morphology is more easily seen in fresh specimens or those preserved in formalin-based fixatives (Fig. 12.10).
4. The eggs are infectious, and unpreserved stool specimens should be handled with caution.

Case 9

A 47-year-old man was admitted to the hospital with complaints of severe mid-epigastric pain that had become worse over a period of about a week. This patient had received prednisone over the course of several years, and the dose had been increased 2 months before. Previous diagnostic testing included an O&P examination, whose results were reported as "No parasites seen."

He was treated with supportive care for epigastric pain and developing pneumonia, but his condition failed to improve. He became comatose and died 3 days later. Autopsy findings included the image (of his colon) in Fig. 12.11.

Questions

1. What infection most probably matches this image?
2. Based on the history, why might there be no evidence of eosinophilia?
3. What was there about his history that could lead to complications with this parasite?
4. Does the negative O&P examination in this case make sense? Why or why not? Should a repeat O&P examination have been ordered?
5. Which other test could have been ordered? Is this test more or less sensitive that the routine O&P examination?

Correct Identification

The autopsy image shows a *Strongyloides stercoralis* rhabditiform larva in a crypt of the colon. Remember, high eosinophilia may or may not be present. In this case, no eosinophils were noted (eosinopenia can occur in the hyperinfection syndrome and is a poor prognostic sign). Additional images can be seen in Fig. 12.12.

This case represents a generalized infection with *S. stercoralis*, involving the lungs, liver, peritoneum, small intestine, colon, respiratory diaphragm, heart, lymph nodes, skeletal muscle, and periadrenal and peripancreatic fat. There was generalized peritonitis. Intact rhabditiform and filariform larvae of *S. stercoralis* were seen in the tissues and in stool specimens. It is unfortunate that addi-

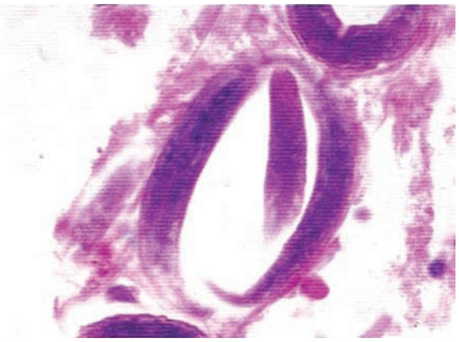


Figure 12.11 Case 9. Image from autopsy (colon). (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch12.f11

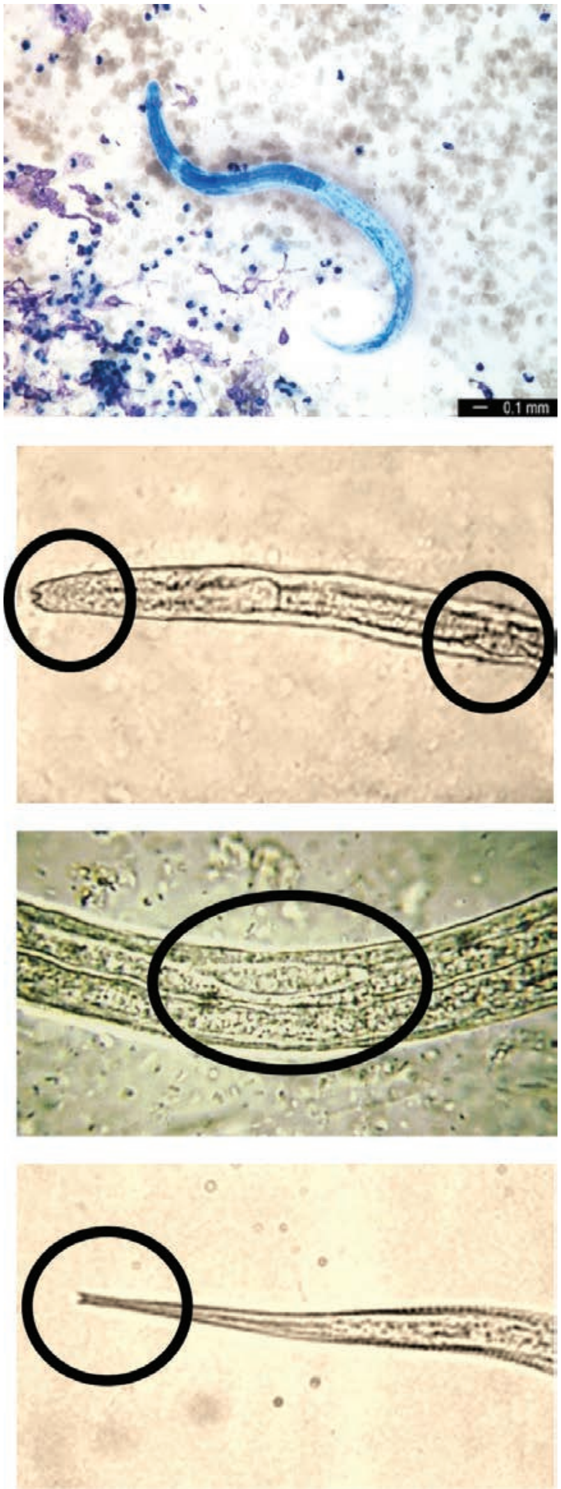


Figure 12.12 Case 9. *Strongyloides stercoralis*. (Top) Larvae in sputum; (second from top) short buccal capsule and genital primordium packet of cells in a rhabditiform (noninfective) larva; (third from top) clear image of the genital primordium packet of cells in a rhabditiform larva; (bottom) slit in the tail of filariform (infective) larva obtained using the Baermann concentration apparatus. doi:10.1128/9781555819002.ch12.f12

tional O&P examinations or the agar plate culture were not performed during this hospital admission.

Strongyloidiasis is most often found in warm climates but can also occur in temperate and cold climates. It overlaps the same geographic range as hookworm infections. The disease is contracted by penetration of the filariform larvae into the skin by contact with infected soil, by autoinfection, or by fecal-oral contamination. Generalized disease may present in many ways: paralytic ileus, an acute surgical abdomen, asthma, and as a protein-losing enteropathy with malabsorption. It should be considered in the differential diagnosis of patients with bronchopneumonia and accompanying abdominal symptoms.

Findings of bilateral pulmonary disease were due to extensive intra-alveolar hemorrhage and interstitial pneumonia; numerous larval forms were present in the lungs. When young worms break out of the pulmonary capillary into the alveoli, hemorrhage and cellular infiltration into the air sacs and bronchioles result.

Malnutrition, lymphoma, treatment with immunosuppressive agents, and corticosteroid therapy are predisposing factors for strongyloidiasis. It is also very important to consider this infection in military personnel and travelers who may have been in an area of endemic infection many years earlier. More than 30 to 40 years after acquisition of the original infection, persistent, undiagnosed disease can be found in these individuals. If, for any reason, they become immunocompromised, the result can be disseminated disease leading to the hyperinfection syndrome and death.

In situations in which autoinfection occurs, some of the rhabditiform larvae that are within the intestine develop into the filariform larvae while passing through the bowel. These larvae can then reinfect the host by (i) invading the intestinal mucosa, traveling via the portal system to the lungs, and returning to the intestine, or (ii) being passed out in the feces and penetrating the host on reaching the perianal or perineal skin (12, 13).

Pathology present in strongyloidiasis can vary both in severity and in the areas of the body involved. Some individuals may remain totally asymptomatic, with the only abnormal clinical finding being a peripheral eosinophilia.

Cutaneous Infection. Initial skin penetration usually causes very little reaction, although there may be some pruritus and erythema if there are large numbers of penetrating larvae. With repeated infections, the patient may mount an allergic response that prevents the parasite from completing the life cycle. The larvae may be limited to skin migration or larva migrans. The term larva currens ("racing larvae") was proposed in 1958 and is now generally accepted for cases of strongyloidiasis in which one or more rapidly progressing linear urticarial tracks starting near the anus are found. There is speculation that some of these cases may involve larvae of other species of *Strongyloides*. These tracks may progress as fast as 10 cm/h, with intermittent movement, usually on the thighs. Onset is sudden, and the lesions may disappear within 12 to 18 h.

Pulmonary Infection. Larval migration through the lungs may stimulate symptoms, depending on how many larvae are present and the intensity of the host immune response. Some patients are asymptomatic, while others present with pneumonia. With a heavy infective dose or in the hyperinfection syndrome, individuals often develop cough, shortness of breath, wheezing, fever, and transient pulmonary infiltrates (Loeffler's syndrome). Cases have also been reported where the larvae can be found in the sputum (Fig. 12.12).

Intestinal Infection. In heavy infections, the intestinal mucosa may be severely damaged with sloughing of tissue, although this type of damage is unusual. Symptoms may mimic peptic ulcer with abdominal pain, which may be localized in the right upper quadrant. Radiographic findings may mimic Crohn's disease of the proximal small intestine. In immunocompetent patients there is a leukocytosis with a peripheral eosinophilia of 50 to 75%, while in chronic cases the eosinophilia may be much lower. Some of these chronic infections have lasted for over 30 years as a result of the autoinfective capability of the larvae. One case of chronic strongyloidiasis persisted for approximately 65 years.

Hyperinfection Syndrome. Autoinfection is probably the mechanism responsible for long-term infections that persist for years after the person has left the area of endemic infection. The parasite and host reach a status quo so that neither suffers any serious damage. If for any reason this equilibrium is disturbed and the individual becomes immunosuppressed, then the infection proliferates, with large numbers of larvae being produced and found in every tissue of the body. Several conditions, including the use of immunosuppressive therapy, predispose an individual to the hyperinfection syndrome. In addition to the actual tissue damage from the migrating larvae, the patient may die from sepsis, primarily as a result of the intestinal flora. Other causes of death include peritonitis, brain damage, and respiratory failure.

Reactive Arthritis. Few cases of reactive arthritis have been reported, and the number of cases may be underestimated. A case of reactive arthritis combined with uveitis associated with a long-standing, heavy *Strongyloides* infection has been found in a 32-year-old patient infected with human T-cell leukemia virus. Treatment with thiabendazole and ivermectin resulted in rapid improvement.

Debilitated or compromised patients should always be suspected of having strongyloidiasis, particularly if there are unexplained bouts of diarrhea and abdominal pain, repeated episodes of sepsis and/or meningitis with intestinal bacteria, or unexplained eosinophilia. However, a recent comparative study of the occurrence of *Strongyloides* in 554 AIDS and 142 non-AIDS patients demonstrated a similar prevalence of infection in the two groups, indicating that there are no significant statistical differences.

Key Points for Laboratory Identification

1. The rhabditiform (noninfective) larvae are normally recovered from the stool concentrate. However, it is important to remember that filariform (infective) larvae can also be recovered in the stool.
2. If the stool specimens are negative, examination of duodenal contents is recommended (duodenal aspirates, Entero-Test capsule).
3. Various concentrates (Baermann) and cultures (Harada-Mori, petri dish, or agar plate) can also be used for larval recovery.
4. Eggs are rarely seen in the stool but may be recovered from duodenal contents.
5. In very heavy infections, eggs (less common), larvae (both types), and adult worms may be recovered in the stool.
6. Remember that if agar plates are used, they must be dried at room temperature for 4 to 5 days to eliminate free water on the agar prior to use. The agar plate culture method is thought to be the most sensitive diagnostic procedure available (2–5); it is also considered somewhat cumbersome to perform.

Thiabendazole has been used in the past; in some cases, repeated or extended (1 week) therapy may be necessary, and the cure rates range from 55 to 100%. Patients with the hyperinfection syndrome should be hospitalized during therapy for proper monitoring. Ivermectin has become more widely used in the last few years (13).

Contact with contaminated infective soil, feces, or surface water should be avoided. Individuals found to have the infection should be treated. All patients who are going to receive immunosuppressive drugs should be screened for strongyloidiasis before therapy.

Strongyloidiasis is endemic at least as far north as New York City. It is very likely that endemic foci also exist in other metropolitan areas within the United States, and cases similar to the one presented here could occur anywhere within the country. This was a case of disseminated strongyloidiasis presenting as acute abdominal distress in an adult who had been receiving immunosuppressive therapy.

Case 10

A 34-year-old man from Mexico was seen in the Tropical Medicine Clinic because he found some white, rectangular objects in his stool. These objects were about an inch long and appeared to be "moving" very slowly. He had always been healthy and had come to the United States 2 years earlier. He denied experiencing gastrointestinal or other symptoms. He had lived in a rural area of Mexico, and he reported no unusual dietary habits or significant contact with animals. During the previous few days, he complained of anorexia, some nausea, and abdominal cramps, but no diarrhea. Laboratory findings were normal; however, the routine O&P examination revealed the images shown in Fig. 12.13 (2–5).

Questions

1. What do you think the structures are as seen in Fig. 12.13?
2. Are there any key morphologic characteristics that could help you identify this parasite?
3. What laboratory tests might be appropriate at this point?
4. Are there any standard precautions that should be taken? If so, what are they and why might they be important? If not, why not?

Correct Identification

The helminth eggs are those of *Taenia* spp; note the heavily striated shell and the hooklets within the oncosphere (embryo within the shell). From the egg morphology, it is impossible to identify the cestode to the species level as *T. saginata* (beef tapeworm) or *T. solium* (pork tapeworm). This structure recovered from the patient's stool specimen is a *Taenia* sp. proglottid. To more fully visualize the internal structure of the proglottid, an India ink injection procedure could be performed. Results of this procedure can be seen in Fig. 12.14.

The life cycle of *T. saginata* is very similar to that of *T. solium*. Infection with the adult worm is initiated by the ingestion of raw or poorly cooked beef containing encysted *T. saginata* larvae. As with *T. solium*, the larva is digested out of the meat in the stomach and the tapeworm evaginates in the upper small intestine and attaches to the intestinal mucosa, where the adult worm matures within 5 to 12 weeks. The adult worm can reach a length of 25 m but often measures only about half this length. Although a single worm is usually found, multiple worms can be present (personal observation).

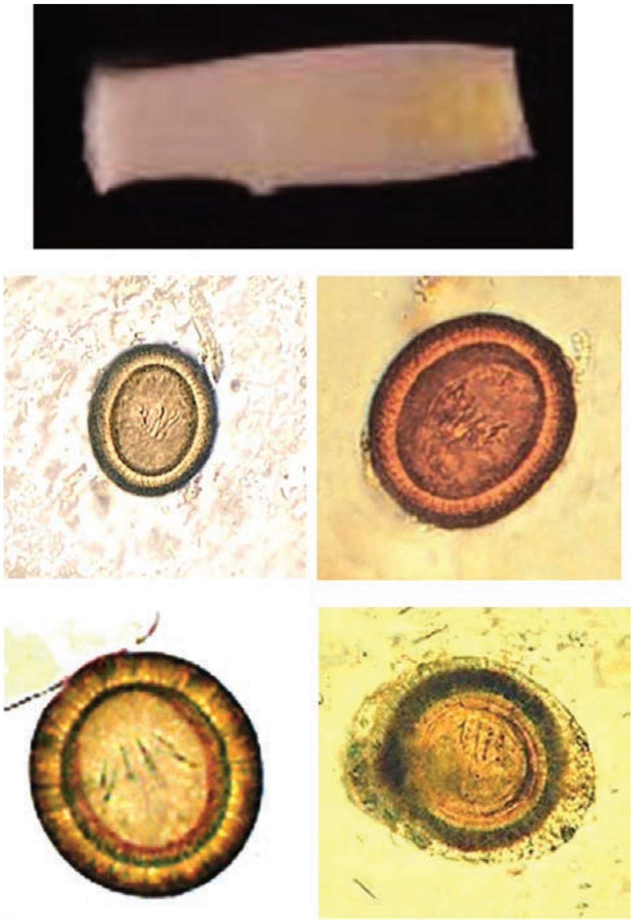


Figure 12.13 Case 10. (Top) Structure obtained from stool specimen; (lower four images) objects seen in the concentration sediment wet mount (using high dry 40× objective); second row, left is photographed at a lower magnification. (Bottom row, left, courtesy of Yanagida T et al., *Parasites & Vectors* **5**:18, http://www.parasitesandvectors.com/content/5/1/18 [accessed 9/14/2013].) doi:10.1128/9781555819002.ch12.f13

The scolex is "unarmed" and has four suckers with no hooks. The proglottids usually number 1,000 to 2,000, with the mature proglottids being broader than long and he gravid proglottids being narrower and longer. Identification to the species level is usually based on the number of main lateral uterine branches, which are counted on one side of the gravid proglottid. There are between 15 and 20 branches, with an average of 18.

Gravid proglottids often crawl from the anus during the day, when the host is most active. These proglottids may crawl under the stool when a specimen is submitted unpreserved in the stool collection carton. The eggs cannot be distinguished from those of *T. solium*. They are round to slightly oval, measure 31 to 43 µm, have a thick, striated shell, and contain the six-hooked embryo (oncosphere). The eggs can remain viable in the soil for days to weeks.

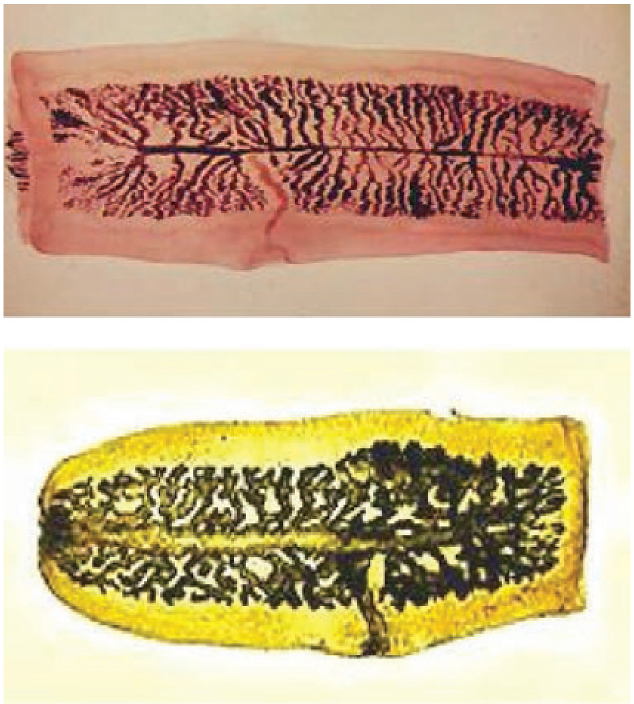


Figure 12.14 Case 10. *Taenia saginata* gravid proglottids; note the large number of lateral uterine branches when counted on one side. doi:10.1128/9781555819002.ch12.f14

On ingestion by cattle, the oncospheres hatch in the duodenum, penetrate the intestinal wall, and are carried via the lymphatics or bloodstream, where they are filtered out in the striated muscle. They then develop into the bladder worm or cysticercus within approximately 70 days. The mature cysticercus measures 7.5 to 10 mm wide by 4 to 6 mm long and contains the immature scolex, which has no hooks (unarmed). Other animals found to harbor cysticerci include buffalo, giraffe, llama, and possibly reindeer. Actual cases of human cysticercosis with *T. saginata* are rare in the literature, and there is speculation that some reported cases have been inaccurately diagnosed.

There are usually few symptoms associated with the presence of the adult worm in the intestine. Although rare symptoms (obstruction, diarrhea, hunger pains, weight loss, and appendicitis) have been reported, the most common complaint is the discomfort and embarrassment caused by the proglottids crawling from the anus. This occurrence may be the first clue that the patient has a tapeworm infection. Occasionally, the proglottids are also seen on the surface of the stool after it is passed.

Key Points for Laboratory Identification

1. Preliminary examination of the gravid proglottid may not allow identification without clearing or injection of the uterine branches with India ink. Since there is always the possible danger that the proglottid is *T. solium*, with the inherent problem of egg ingestion and cysticercosis, all specimens should be handled with extreme caution.
2. The eggs look identical to those of *T. solium*, so that identification to the species level cannot be performed with eggs alone unless special staining is performed. Ziehl-Neelsen staining can occasionally distinguish fully mature *T. solium* from *T. saginata* eggs, but this distinction is neither very sensitive nor completely specific. *T. saginata* eggs tend to stain magenta, while *T. solium* eggs tend to stain blue/purple.
3. Although gravid proglottids of *T. saginata* are often somewhat larger than those of *T. solium*, this difference may be very minimal or impossible to detect.
4. Any patient with *Taenia* eggs recovered in the stool should be cautioned to use good hygiene. These patients should be treated as soon as possible to avoid the potential danger of accidental infection with the eggs, which may lead to cysticercosis (*T. solium*).

For therapy, the use of praziquantel or niclosamide has been recommended. The same general approach as used for *T. solium* is used to eradicate the adult worm. Therapy is usually very effective; however, if the proglottids begin to reappear in the stool or crawl from the anus, retreatment is necessary.

Case 11

A 45-year-old engineer presented to his physician with jaundice. He reported that a few days earlier he had developed a fever and also had experienced diarrhea, nausea, and vomiting. He also indicated that he had had a cough about a week earlier. A few days later, he experienced right upper quadrant pain. On the day he presented to the physician, he had noticed that his eyes were somewhat yellow. The patient works for an American-based engineering firm that has multiple contracts throughout South America; their main responsibilities include drilling for water and construction of irrigation canals in recently cleared land areas of several South American countries, including Bolivia, Argentina, Chile, and Peru. Periodically he spends time in these areas, usually eating whatever is available. Often the engineering job is adjacent to sheep- and/or cattle-raising regions.

On presentation, the patient appeared in no particular distress; however, he was jaundiced. The lungs and heart were normal. There was guarding and tenderness in the right upper quadrant. The liver was enlarged and tender down to 3 cm below the costal margin, with a span of 15 cm. There was no peripheral edema. The white blood cell count was 18,500, with 35 polymorphonuclear leukocytes, 10 lymphocytes, 3 monocytes, and 52 eosinophils. Stool examinations for parasites revealed the image shown in Fig. 12.15. These objects measured about 140 by 70 µm and were easily seen in the saline/iodine wet preparation examination.

Questions

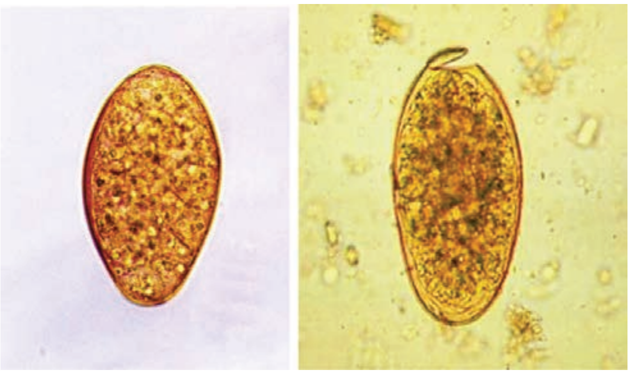
1. Based on the size of the objects in Fig. 12.15, what are the possibilities regarding identification?
2. What key morphologic features are helpful in making the diagnosis?
3. Are these key characteristics visible? If yes, what are they? If not, why not?
4. Are there any additional laboratory tests that might be helpful?

Correct Identification

The object in Fig. 12.15 is a *Fasciola hepatica* egg; these eggs generally measure 130–150 by 63–90 µm. They are unembryonated, operculated, large, ovoid, and usually brownish yellow (2). However, based on morphology alone, the egg could also be *Fasciolopsis buski* or *Fasciola gigantica*. Note the open operculum seen in the image on the right.

The patient's symptoms reflect the phase of the infection, as well as the number of parasites present in the host. In the acute phase, symptoms may be present over a period of weeks to months. Metacercarial larvae do not produce significant pathologic damage until they begin to migrate through the liver parenchyma. The amount of damage depends on the worm burden of the host. Linear lesions of 1 cm or greater can be found. Hyperplasia of the bile ducts occurs, possibly as a result of toxins produced by the larvae. Symptoms associated with this migratory phase can include fever, epigastric and right upper quadrant pain, and urticaria. Asymptomatic infections appear to be more common in Peru.

Figure 12.15 Case 11. Objects seen in the wet mount microscopic examination of concentration sediment. These objects measured about 140 by70 µm and were easily seen in the saline/iodine wet preparation examination.
doi:10.1128/9781555819002.ch12.f15



In the more chronic phases of the disease, the patient generally has few to no symptoms once the flukes have lodged in the biliary passages. However, there may be some epigastric and right upper quadrant pain, diarrhea, nausea, vomiting, hepatomegaly, and jaundice. If the flukes are found in the extrahepatic biliary ducts, symptoms may mimic those seen in cholelithiasis. In the chronic phase, there tend to be some liver function abnormalities, as well as eosinophilia.

Once the worms have established themselves in the bile ducts and matured, they cause considerable damage from mechanical obstruction and metabolic by-products. The degree of pathologic change depends on the number of flukes penetrating the liver. The infection produces hyperplasia of the biliary epithelium and fibrosis of the ducts, with portal or total biliary obstruction. The gallbladder undergoes similar pathologic changes and may even harbor adult worms.

Symptoms and signs of infection during the late stages after egg production has begun are those associated with biliary obstruction and cholangitis. Acute epigastric pain, fever, pruritus, jaundice, hepatomegaly, and eosinophilia are common.

Adult worms, which may live for 9 years in the bile ducts, produce eggs that are carried by the bile fluid into the intestinal lumen and passed into the environment with the feces. The eggs are unembryonated, operculated, large, ovoid, and brownish yellow. The miracidium develops within 1 to 2 weeks and escapes from the egg to infect the snail intermediate host, *Lymnaea* sp. Cercariae are liberated from the snail after the production of a sporocyst generation and two or three rediae generations. Cercariae encyst on water vegetation, e.g., watercress. Humans are infected by ingestion of uncooked aquatic vegetation on which metacercariae are encysted. Metacercariae excyst in the duodenum and migrate through the intestinal wall into the peritoneal cavity. The larvae enter the liver by penetrating the capsule (Glisson's capsule) and wander through the liver parenchyma for up to 9 weeks. They finally enter the bile ducts, where they mature and produce eggs, which are passed out in feces. The adult worms can attain a length of >1 in. (1 in. = 2.54 cm) and a width of about 0.5 in.

Fascioliasis is a cosmopolitan disease found where there is close association of livestock, humans, and snails. The largest numbers of infections have been found in Bolivia, Ecuador, Egypt, France, Iran, Peru, and Portugal. Although animal fascioliasis is endemic throughout the Americas, human infections are rare in most countries. Reservoir hosts include herbivores such as cattle, goats, and sheep. Animal fascioliasis is a major veterinary problem in Europe. Watercress is the main source of human infection, and in many countries wild watercress is touted as a healthy natural food. In many areas of the world, animal manure is used as the primary fertilizer for cultivation of watercress.

Prevention may be accomplished by public health education in areas where infections are endemic, stressing the dangers of eating watercress grown in the wild where animals and snails are abundant. Other measures have included mollusciciding, treatment of infected animals, and draining of pasture lands.

Key Points for Laboratory Identification

1. Multiple stool specimens may be required to find the trematode eggs. In a very light infection, eggs may not be recovered; however, the patient might be asymptomatic.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method. Note the open operculum seen in the egg on the right.
3. When adding iodine to the wet preparation, do not add too much or the eggs will stain very darkly and resemble debris.
4. The wet preparation can be examined using the 10× (low-power) objective; these eggs are large enough that they can usually be seen using this magnification.
5. Eggs of *F. hepatica* can resemble those of *Fasciola gigantica* (liver fluke) and *Fasciolopsis buski* (intestinal fluke).
6. Do not make the wet preparation too thick; if it is too thick, the eggs can be obscured by normal stool debris.
7. If available, antigen or antibody detection may be helpful in confirming the infection.

Although treatment with praziquantel at a dose of 25 mg/kg taken after each meal for 2 days is sometimes effective, it does not appear to be effective in treating cases of infection in Egypt. Treatment with 30 to 50 mg of biothionol/kg on alternate days for 10 to 15 doses is recommended. Triclabendazole at 10 mg/kg as a single dose is also recommended but may not be as easily obtained. The drug is given orally in single or multiple doses and has few side effects. The drug acts by inhibiting protein synthesis in *F. hepatica* and will probably become the drug of choice.

Case 12

A 14-month-old boy was admitted to the hospital with complaints of irritability, ataxia, and weakness that had developed over a period of several weeks. Just prior to admission, he was unable to sit up or walk. A complete blood count showed 35% eosinophilia; lumbar puncture revealed an elevated eosinophilia. His neurologic status did not improve. Several weeks after admission, his magnetic resonance imaging scan revealed severe cortical atrophic changes and severe diffuse white-matter degeneration. He remained in a vegetative state and died several months after his initial symptoms. He was not seropositive for *Toxocara canis*, and he had not lived in or traveled to areas where other causes of eosinophilic meningoencephalitis would be suspected. At autopsy, the image in Fig. 12.16 was observed in brain tissue (14; http://wwwnc.cdc.gov/eid/article/8/4/01-0273_article.htm).

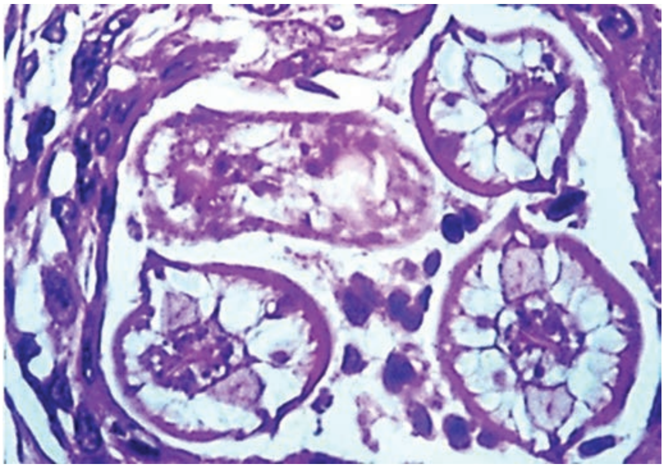
Questions

1. What are some of the other causes of eosinophilic meningoencephalitis?
2. What questions would you ask regarding possible patient history details (animal contact, play practices, etc.)?
3. Does the patient's age suggest any possible diagnostic options?
4. What preventive measures could be taken to avoid this particular infection?

Correct Identification

The image seen in Fig. 12.16 reveals a histologic section of larvae consistent with the raccoon roundworm, *Baylisascaris procyonis*. On further questioning, it became clear that the child had been exposed to feral raccoons and soil contaminated with raccoon feces in his backyard. Raccoons were seen in the surrounding area, and typical *B. procyonis* eggs were found in the soil. Adult worms were

Figure 12.16 Case 12. Image from autopsy (brain tissue). (From Sorvillo F et al, *Emerg Infect Dis* 8, 2002; http://wwwnc.cdc.gov/eid/article/8/4/01-0273-f2.)
doi:10.1128/9781555819002.ch12.f16



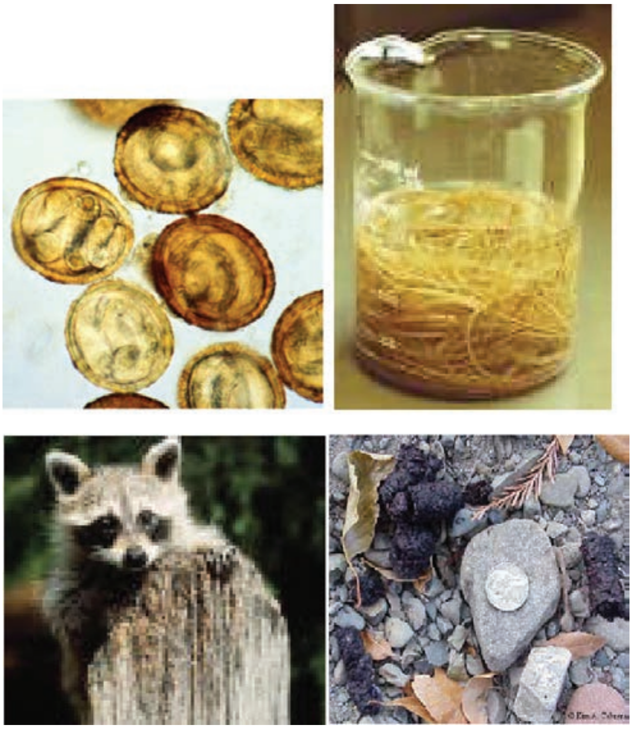
also found in the raccoons (Fig. 12.17); the adult worms look very much like *Ascaris lumbricoides*.

The diagnosis of *B. procyonis* encephalitis was confirmed. This patient presented with the typical clinical syndrome, including peripheral and CSF eosinophilia. On further testing, the patient demonstrated positive antibody results in indirect-immunofluorescence testing of both serum and CSF to *B. procyonis*. Both the intensity and titers increased with time. Other infections causing eosinophilic meningoencephalitis include *Toxocara* spp. larval migrans.

Key Points for Laboratory Diagnosis

1. Definitive diagnosis requires identification of the larvae in tissues; however, this can be difficult depending on the body site. Cross sections of larvae tend to measure 60 to 70 μm, and the larvae have prominent, single lateral alae and paired, conical excretory columns (smaller than central intestine).
2. Ocular examinations may reveal retinal lesions, larval tracks, or migrating larvae; these findings may provide the tentative diagnosis involving a helminth infection.
3. Using indirect fluorescent-antibody testing, enzyme-linked immunosorbent assay, and Western blotting, anti-*Baylisascaris* antibodies can be detected in CSF. Currently, serologic testing is available at CDC, Parasitic Diseases Branch. One of the world's experts is Dr. Kazacos at the Department of Veterinary Pathobiology at Purdue University, West Lafayette, Ind. [K. Kazacos; phone (765) 494-7558]. Acute- and convalescent-phase titers demonstrate several-fold increases in both serum and CSF antibody levels; there is no cross-reactivity with *Toxocara*. With the availability of a reliable serologic test, there is less need to perform a brain biopsy.
4. *In most cases, the clinical history provides the main clues.*

Figure 12.17 Case 12. *Baylisascaris procyonis*. (Upper, left) Typical eggs isolated from raccoons; (right) adult worms isolated from raccoons. (Lower, left) Raccoon; (right) raccoon feces, "raccoon latrine." doi:10.1128/9781555819002.ch12.f17



Feral raccoons were found in the area around the house; both dirt and plant debris were contaminated with viable *B. procyonis* eggs. Ingestion of infective *B. procyonis* eggs probably occurred through ingestion of contaminated soil or other hand-to-mouth transfer from areas or articles contaminated with raccoon feces. This roundworm is a common parasite of raccoons, especially in the Midwest and the Northeast and on the west coast of the United States. Infection rates can range as high as 82% in raccoons. In many metropolitan areas, the raccoon population is quite high, and people tend to lure the animals close to homes through intentional feeding or through dog food left in the surrounding areas. Thus, contamination of the surrounding environment with raccoon feces is common. The infected animals shed an average of >25,000 eggs/g of feces; millions of eggs can be shed each day. These infective eggs are very environmentally resistant and can survive for years in the soil.

Raccoons tend to defecate in the same areas, which are called latrines (often in barns, decks, patios, etc.). These sites remain sources of eggs that are infective for both humans and other animals for long periods. Young children with a history of ingesting soil and/or feces are at greatest risk for infection; most confirmed cases have been in this age group. Individuals with developmental impairment tend to be at special risk due to a greater tendency to exhibit behaviors that lead to egg ingestion.

Unfortunately, neural larval migrans caused by *B. procyonis* carries a poor prognosis. However, high-dose albendazole given early in the infection may prevent or halt the progression of CNS disease. Treatment before larvae reach the CNS is beneficial. Because they diminish inflammatory reactions, steroids may be beneficial unless the CNS damage is too advanced. Although ivermectin has been used, this drug does not cross the blood-brain barrier.

In addition to direct damage caused by the migrating larvae, it is likely that eosinophil-derived neurotoxin contributes to the manifestations of encephalitis. Two features distinguish this infection from other nematode infections that cause larval migrans: the somatic migration and invasion of the CNS and the continued growth of the larvae to a large size within the CNS. Despite therapy, to date there are no documented cases of neurologically intact survivors.

Although reports of cases of eosinophilic meningoencephalitis caused by *B. procyonis* infections are rare, large populations of raccoons with high rates of *B. procyonis* infections live in close proximity to humans in many areas. Widespread contamination of the domestic environment suggests that the risk of exposure and human infection may be substantial. Prevention of infection is the most important public health measure; educating the public is essential for disease prevention.

There is no effective cure for *B. procyonis* infection; treatment is symptomatic and involves systemic corticosteroids and anthelmintic agents. Unfortunately, neural larva migrans is usually not responsive to anthelmintic therapy; by the time the diagnosis is made, extensive damage has already taken place. Drugs that can be tried include albendazole, mebendazole, thiabendazole, levamisole, diethylcarbamazine, and ivermectin. Experimental data from animal studies indicate that albendazole and diethylcarbamazine may have the best CSF penetration and larvicidal activity. Unfortunately, since the diagnosis does not take place until symptoms have begun, larval invasion of the CNS has already taken place. Therefore, treatment is usually started too late in the course of the infection. However, rapid presumptive treatment with albendazole and high-dose steroids may halt progression of clinical signs (15).

Considering the potential outcomes associated with delay of treatment until symptoms begin, prophylactic treatment for asymptomatic children exposed to raccoons or contaminated environments is now being considered. Since the anthelmintics do not appear to be problematic, prophylaxis seems appropriate for specific individuals. Albendazole has been used in these cases; treatment has been discontinued when environmental testing has proven to be negative.

Case 13

A 33-year-old female, originally from Mexico, was admitted to the hospital with seizures. This patient had previously experienced convulsions when she was 14, but had remained asymptomatic until about a year prior to admission. The seizures had become more numerous and now occurred about once a month. The patient had experienced an inability to talk and had become unconscious for several minutes during these episodes. She had also complained of double vision.

On admission, the patient appeared to have partial paralysis on the right side and complained of progressive weakness, again on the right side. Computed tomographic and magnetic resonance imaging studies revealed several intracerebral lesions, some of which appeared to be calcified. On surgery, these structures (Fig. 12.18) were seen and removed. The gross specimen measured 3 × 4 cm.

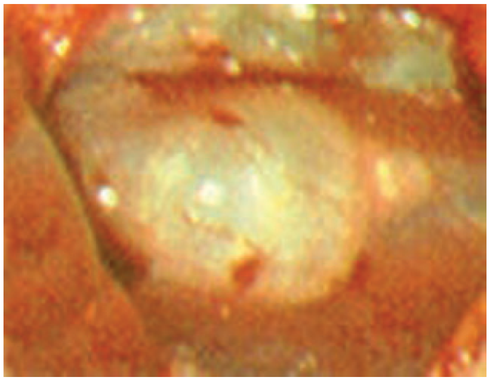


Figure 12.18 Case 13. Gross specimen (intracerebral lesion), measuring 3 by 4 cm. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch12.f18

Routine pathology revealed the images seen in Fig. 12.19.

Questions

1. What organisms might be present in the images shown?
2. What did the patient ingest in order to become infected?
3. In individuals from Mexico and Central and South America, this parasite should be suspected in patients who present with what symptoms?

Correct Identification

Parasites that come to mind include *Taenia solium* (pork tapeworm—cysticercosis) or *Echinococcus granulosus* (sheep/sheep dog tapeworm—hydatid disease). The patient must have ingested some viable eggs of *T. solium*. The eggs hatch, and the larvae migrate into the body tissues. When the larvae die, they calcify and are visible radiographically. Viable cysts can be visualized using various scan methods or magnetic resonance imaging techniques (lesions in the brain). Any patient from these geographic areas who presents with seizures should be considered for possible cysticercosis.

The gross specimen (Fig. 12.18) is a *T. solium* cysticercus that has been removed from the brain. Tissue sections represent a *T. solium* cysticercus that consists of a fluid-filled bladder containing a single protoscolex (Fig. 12.19). Each cysticercus measures approximately 5 to 15 mm long by 4 to 12 mm wide (although some may be somewhat

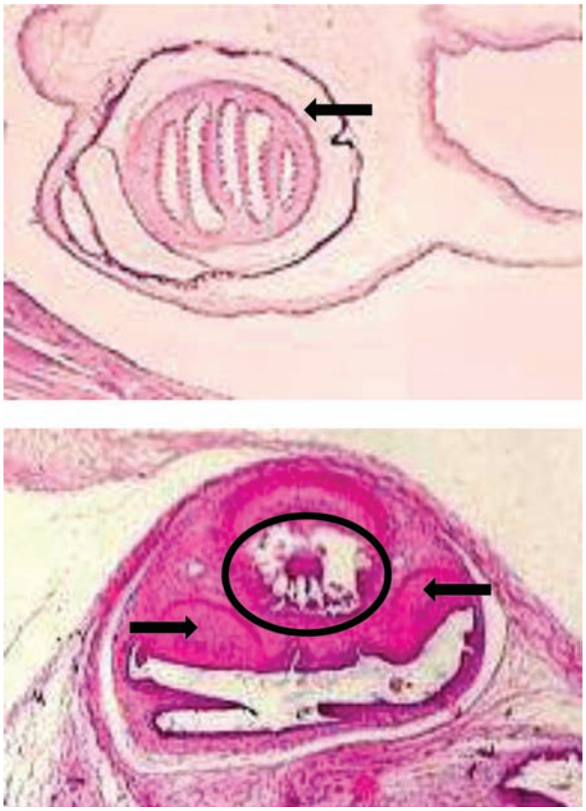


Figure 12.19 Case 13. Routine pathology preparations from tissue seen in Fig. 12.18. doi:10.1128/9781555819002.ch12.f19

smaller). A convoluted spiral canal leads to the rostellum, and the parenchyma usually contains calcareous corpuscles. The image on the right is a tissue section of a *T. solium* cysticercus in the brain, surrounded by fibrous tissue. The invaginated scolex is present; however, the four suckers and hooklets may not always be visible.

This case represents an infection with *T. solium*, the cause of cysticercosis. When these larvae are found in the brain, symptoms can result from actual larval invasion of the brain tissue and/or death or the organism, which stimulates tissue reactions to the larvae. It has been recommended that in every case of epilepsy occurring in a patient with no family history of seizures and no previous history of seizures in childhood, the probability of cysticercosis should be considered. Other symptoms, including abnormal behavior, transient paresis, intermittent obstructive hydrocephalus, disequilibrium meningoencephalitis, and visual problems, may also be present. In this patient there was a history of seizures in childhood, and many of the key symptoms of cysticercosis were also present.

The cysticercus is an ovoid, milky white bladder with the head invaginated into the bladder. The bladder worms measure 5 mm long by 8 to 10 mm wide, except in brain or submeningeal spaces where they can have a volume of about 60 ml. The larval forms of *T. solium* (cysticerci) have been recovered from all areas of the body, and symptoms depend on the body site involved. The presence of cysticerci in the brain represents the most frequent parasitic infection of the human nervous system and the most common cause of adult-onset epilepsy throughout the world.

On ingestion by hogs or humans, the eggs of *T. solium* hatch in the duodenum or jejunum after exposure to gastric juice in the stomach. The released oncospheres penetrate the intestinal wall, are carried via the mesenteric venules throughout the body, and are filtered out in the subcutaneous and intramuscular tissues, the eyes, the brain, and other body sites. Neurocysticercosis usually affects males and females of all ages, with a peak incidence between 30 and 50 years of age.

Key Points for Laboratory Diagnosis

1. The diagnosis of cysticercosis usually depends on surgical removal of the parasite and microscopic examination for the presence of suckers and hooks on the scolex. Routine histology can also be performed.
2. Multiple larvae are frequently present, and the presence of cysticerci in the subcutaneous or muscle tissues indicates that the brain is probably also involved.
3. If the racemose form is present in the brain, the computed tomographic scan will not be able to distinguish the lesion from that caused by other tumors.
4. Although serologic testing is helpful in some cases, there may be cross-reactivity between cysticercosis and hydatid infection (*Echinococcus* spp.), and the results may be erratic. The immunoblot for neurocysticercosis provides a much improved test, with a sensitivity of 98% and a specificity of 100%. The enzyme-linked immunoelectrotransfer blot (EITB) assay using blood dried on filter paper has proven to be a convenient, economical way of handling field samples, particularly in underdeveloped countries. There is no decrease in sensitivity compared with traditional serum samples used in the EITB.

Cysticerci have been recovered from all areas of the body, and symptoms depend on the particular body site involved. The presence of cysticerci in the brain represents the most frequent parasitic infection of the human nervous system and the most common cause of adult onset epilepsy throughout the world (16, 17). For unknown reasons, in Latin America it is unusual for both brain and muscle cysticercosis to occur in the same patient, with fewer than 6% of patients having cysticerci in both sites. However, elsewhere in the world, subcutaneous involvement by *T. solium* cysticerci has been reported in as many as 78.5% of patients with cerebral cysticercosis (18). Possible reasons for such differences may include (i) the immune status of the patient, (ii) the human leukocyte antigen type, (iii) the nutritional status of the patient, (iv) the burden of eggs infecting the patient, and (v) a difference in the strains of *T. solium*. It is also rare for a patient with cysticercosis to harbor adult *T. solium* worms in the intestine. Neurocysticercosis usually affects males and females of all ages, with a peak incidence between 30 and 50 years of age.

Neurocysticercosis is the most common parasitic disease of the CNS; in some countries the prevalence exceeds 10% and is responsible for up to 50% of late-onset epilepsy cases. Factors related to neurocysticercosis pathology include (i) the individual immune response to the presence of the parasite, from tolerance to a severe inflammatory response; (ii) the location of the parasite in the brain; (iii) the number of cysticerci present; and (iv) the stage or age of the lesions (live cysts, inflammatory exudates, granulomas, calcifications, and residual fibrosis).

Case 14

A 67-year-old female presented to her primary care physician with right upper quadrant pain. She had a history of frequent foreign travel, including a number of countries in South America and Asia. Imaging demonstrated a 12.5-cm hepatic cyst. The cyst was drained and subsequent examination of the fluid indicated no infectious or malignant etiology. Serology tests for *Entamoeba histolytica* (amebiasis) and *Echinococcus* (hydatid disease) antibodies were negative. The patient underwent additional hepatic cyst aspirations for recurring symptoms over a number of years. Approximately 4 years later, imaging demonstrated cyst enlargement and some more solid internal components within the cyst. Repeat *Echinococcus* antibodies ordered were elevated. Cyst aspiration demonstrated the images seen in Fig. 12.20.

Questions

1. What parasitic infections should be considered in the diagnosis?
2. Do the images in Fig. 12.20 provide a diagnosis?
3. Is her travel history relevant? Why or why not?
4. Why didn't the original cyst drainage demonstrate parasitic elements?

Correct Identification

Based on the images seen in Fig. 12.20, the presumptive diagnosis would be hydatid disease with *Echinococcus granulosus*; hooklets from disintegrated protoscolices are visible, as are some of the intact protoscolices. Currently, four species are recognized within the genus *Echinococcus*: *E. granulosus* (which causes cystic disease), *E. multilocularis* (which causes alveolar disease), *E. vogeli* (which causes polycystic disease), and *E. oligarthrus* (which causes polycystic disease). The areas of the world involved in sheep and cattle raising tend to be the areas where infections with *E. granulosus* are endemic; they even include the Basque sheep farmers in California. The number of infections in both animals and humans has decreased over the years as a result of education and various control measures. However, in some areas in Central Asia, figures suggest the surgical incidence is now greater than 10/100,000 (up to 27/100,000 in Tajikistan), and many of the cases are in children and the unemployed (19, 20).

The risk of infection depends to a high degree on the association between humans and dogs, with the exception of the lion strain. Those at high risk include populations where dogs are used to herd sheep and are also intimate members of the family, often having unrestricted access to the house and family members. Cystic echinococcosis has been recorded in 21 of China's 31 provinces, autonomous

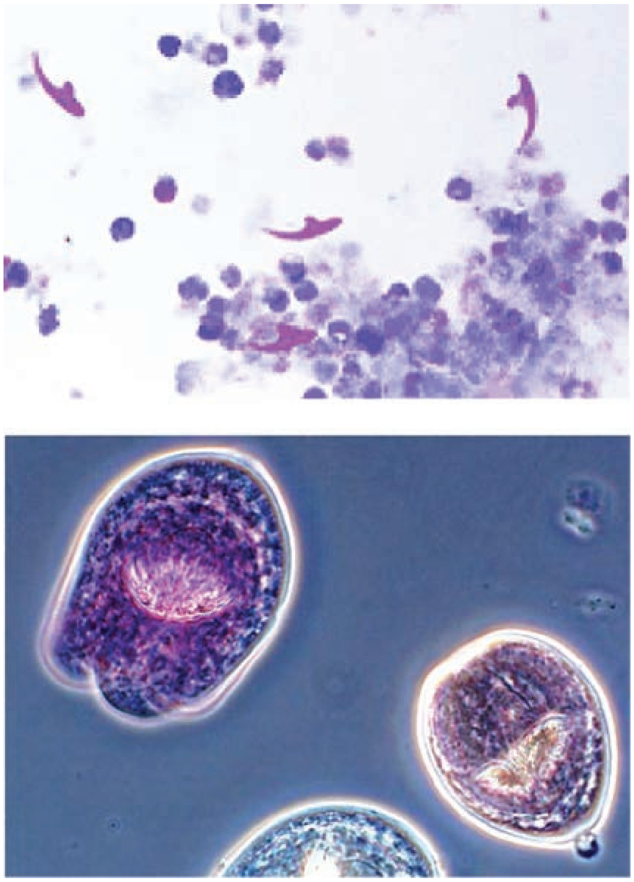


Figure 12.20 Case 14. (Upper) Modified Ryan trichrome stain; (lower) phase-contrast microscopy.
doi:10.1128/9781555819002.ch12.f20

regions, and municipalities (approximately 87% of the territory). This infection constitutes one of the major health problems in this part of the world. Hydatid disease caused by *E. granulosus* is a zoonosis of major public health concern throughout Latin America, particularly in the Andean and South Cone regions. Cystic echinococcosis is also widely found throughout the region comprising Arab North Africa and the Middle East.

After the gravid proglottids and eggs are passed in the feces from the canine host, they may be swallowed by an intermediate host, including humans. After exposure to gastric and intestinal enzymes, the eggs will hatch in the duodenum. The released oncospheres will penetrate the intestine and be carried via the bloodstream, where they will be filtered out in the various organs. The most common site in humans is the liver (60 to 70% of cases). Although not all oncospheres survive this passage through the body, those that do survive begin to grow and develop. Usually by the fifth month, the wall of the hydatid cyst has become differentiated into an outer friable, laminated, nonnucleated layer and an inner nucleated germinal layer. Various daughter cysts (brood capsules) bud off from the inner germinal layer and may remain attached or float free in the interior of the fluid filled cyst. The individual protoscolices bud off from the inner wall of the daughter cysts; these protoscolices and free daughter cysts are called hydatid sand. Each protoscolex will normally invaginate to protect the hooklets. Although not every cyst will produce daughter cysts and/or protoscolices, this general tissue organization is called a unilocular cyst, in which the cyst contents are held within a single limiting cyst wall.

Comments on Human Disease. Hydatid disease in humans is potentially dangerous; however, cyst size and organ location will greatly influence the outcome. Clinical symptoms may appear after an incubation period of several months to years. Hydatid cysts should be considered in patients with abdominal masses with no clearly defined diagnosis. Although eosinophilia is present in 20 to 25% of patients, it is merely suggestive. Many asymptomatic cysts are first discovered after radiologic studies. The cyst will usually have a well-defined margin with occasional fluid level markings. These studies can also be helpful in diagnosing osseous involvement. Scans may also demonstrate a space-occupying lesion, particularly in the liver. If the cyst is large and located in the abdomen, a thrill is sometimes detected.

Once the cyst is discovered and surgical removal is selected as the approach, some of the cyst fluid can be aspirated and submitted for microscopic examination to detect the presence of hydatid sand, thus confirming the diagnosis. Hydatid sand is not always present. Also, if the cyst is old, the daughter cysts and/or protoscolices may have disintegrated, so that only the hooklets are left. These may be difficult to find and identify if there is debris within the cyst.

There are multiple studies in the published literature that reflect large variability in the sensitivity and specificity of serologic tests, including additional variability between laboratories. Also, 10% to 20% of patients with hepatic cysts do not produce detectable specific serum antibodies (IgG) and thus give false-negative results.

Clinical management of hepatic cysts includes albendazole or mebendazole therapy in combination with either surgical resection or the puncture-aspiration-injection-reaspiration (PAIR) procedure.

Key Points for Laboratory Identification

Echinococcus granulosus **(Cystic Disease)**

1. Presumptive diagnosis may be based on history, radiographic studies, or scans.
2. Additional supportive data may be acquired from immunologic tests.
3. Microscopic examination of hydatid cyst fluid may reveal the hydatid sand (protoscolices) or, under certain circumstances, just the hooklets.
4. If the cyst fluid is thick or purulent, it may have to be subjected to a digestion procedure prior to examination; the hooklets will survive this treatment and can then be seen and identified.
5. Light, fluorescence, and epifluorescence microscopy can be used to visualize the hooklets; some approaches will require staining, some will not.

Most human hydatid cysts are probably acquired in childhood and may continue to grow undetected for many years. Several preventive measures are critical. First, all infected viscera from slaughtered animals (intermediate hosts containing hydatid cysts) must be disposed of so that dogs do not have access to this material. Also, personal hygiene must be emphasized to prevent accidental ingestion of the infective eggs from soil contaminated with dog feces, particularly since the eggs are very resistant to disinfectants. In many areas, extensive control measures have also been taken to reduce hydatid disease.

Blood Parasite Infections

Case 15

The patient was a 52-year-old man from California who had just returned from a trip to Africa. About a week after he returned, he began complaining of fever, headache, and general malaise. The first blood specimens examined using automated instrumentation revealed nothing significant. He continued to have high fevers and severe headaches, and eventually several additional blood specimens were submitted to the microbiology laboratory for examination as thick and thin blood films (1–5, 21). The images shown in Fig. 12.21 were seen.

Questions

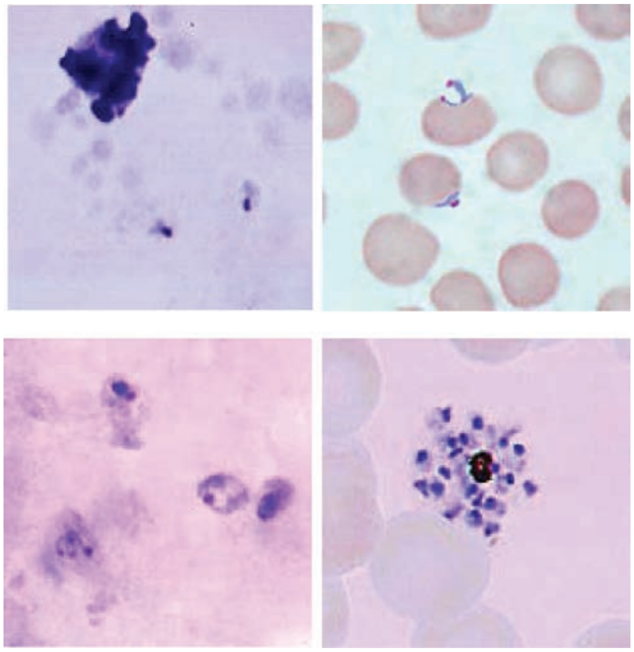
1. What parasitic infections should be considered in the diagnosis?
2. Do the images in Fig. 12.21 provide a diagnosis?
3. Why do you think automation failed to indicate anything significant?
4. Does your presumptive diagnosis match the patient's history? Why or why not?

Correct Identification

At first glance, the fact that the man had just returned from a trip to Africa might suggest a parasitic infection as a possibility. The patient's symptoms were caused by a mixed infection with *Plasmodium falciparum* and *Plasmodium vivax* that he had acquired in Africa. Although he had taken prophylaxis for malaria, he had not taken the medication on a regular basis and did not continue to take the medication after returning to the United States. Because he had never had any contact with malaria before (i.e., he was immunologically naive), he began to have symptoms with a very low parasitemia (0.01%) that was undetectable by automated instrumentation. By the time his symptoms became more severe (about 2 weeks), he had numerous parasites in the peripheral blood. By the time the infection was diagnosed, the man was severely ill. Organisms seen on the blood films were:

1. *P. falciparum* ring form (thick blood film)
2. *P. falciparum* ring forms (thin blood film) (note the typical "headphone" appearance of one of the ring forms)
3. *P. vivax* (thick blood film) (note the developing rings and one developing schizont)
4. *P. vivax* (thin blood film) (note the mature schizont with about 18 merozoites)

Figure 12.21 Case 15. Blood films stained with blood stains. (Upper left) Thick blood film; (right) thin blood film. (Lower left) Thick blood film; (lower right) thin blood film.
doi:10.1128/9781555819002.ch12.f21



Comments on *P. falciparum*. *P. falciparum* tends to invade all ages of RBCs, and the proportion of infected cells may exceed 50%. Schizogony occurs in the internal organs (spleen, liver, bone marrow, etc.) rather than in the circulating blood. Ischemia caused by the plugging of vessels within these organs by masses of parasitized RBCs produces various symptoms, depending on the organ involved. It has been suggested that a decrease in the ability of the RBCs to change shape when passing through capillaries or the splenic filter may lead to the plugging of the vessels.

Onset of a *P. falciparum* malaria attack occurs 8 to 12 days after infection and is preceded by 3 to 4 days of vague symptoms such as aches, pains, headache, fatigue, anorexia, or nausea. The onset is characterized by fever, a more severe headache, and nausea and vomiting, with occasional severe epigastric pain. There may be only a feeling of chilliness at the onset of fever. *The periodicity of the cycle is not established during the early stages, and the presumptive diagnosis may be totally unrelated to a possible malaria infection.* If the fever does develop a synchronous cycle, it is usually a cycle of somewhat less than 48 h. True relapses from the liver do not occur, and recrudescences are rare after a year.

Severe or fatal complications of *P. falciparum* malaria can occur at any time during the infection and are related to the plugging of vessels in the internal organs; the symptoms depend on the organ(s) involved. The severity of the complications in a malaria infection may not correlate with the parasitemia seen in the peripheral blood, particularly in *P. falciparum* infections.

Disseminated intravascular coagulation is a rare complication of malaria; it is associated with high parasite burden, pulmonary edema, rapidly developing anemia, and cerebral and renal complications. Vascular endothelial damage from endotoxins and bound parasitized blood cells may lead to clot formation in small vessels. Cerebral malaria is most often seen in *P. falciparum* malaria, although it can occur in the other types as well. If the onset is gradual, the patient may become disoriented or violent or may develop severe headaches and pass into a coma. Some patients, even those who exhibit no prior symptoms, may suddenly become comatose. Physical signs of CNS involvement are quite variable, and there is no real correlation between the severity of the symptoms and the peripheral blood parasitemia.

Although malaria is no longer endemic within the United States, this infection is considered to be life-threatening, and laboratory requests for blood smear examination and organism identification should be treated as STAT requests. Malaria is usually associated with patients having a history of travel within an area where malaria is endemic, although other routes of infection are well documented. During 2002 (the most recent data available), >45% of the malaria cases reported in the United States were caused by *P. falciparum*, the most pathogenic of the four species infecting humans.

Comments on *P. vivax*. The primary clinical attack usually occurs 7 to 10 days after infection, although there are strain differences, and a much longer incubation period is possible. In some patients, symptoms such as headache, photophobia, muscle aches, anorexia, nausea, and sometimes vomiting occur before organisms can be detected in the bloodstream. In other patients, the parasites can be found in the bloodstream several days before symptoms appear.

During the first few days, the patient may not exhibit a typical paroxysm pattern but, rather, may have a steady low-grade fever or an irregular remittent fever pattern. Once the typical paroxysms begin, after an irregular periodicity, a regular 48-h cycle is established. An untreated primary attack may last from 3 weeks to 2 months or longer. The paroxysms become less severe and more irregular in frequency and then stop altogether. In 50% of patients, relapses occur after weeks, months, or up to 5 years (or more).

Severe complications are rare in *P. vivax* infections, although coma and sudden death or other symptoms of cerebral involvement have been reported. These patients can exhibit cerebral malaria, renal failure, circulatory collapse, severe anemia, hemoglobinuria, abnormal bleeding, acute respiratory distress syndrome, and jaundice. Studies have confirmed that these were not mixed infections with *P. falciparum* but single-species infections with *P. vivax*. However, of the two infections, *P. falciparum* causes the most severe complications.

Since *P. vivax* infects only the reticulocytes, the parasitemia is usually limited to around 2 to 5% of the available RBCs. Splenomegaly occurs during the first few weeks of infection, and the spleen progresses from being soft and palpable to hard, with continued enlargement during a chronic infection. If the infection is treated during the early phases, the spleen will return to its normal size.

Leukopenia is usually present; however, leukocytosis may be present during the febrile episodes. Concentrations of total proteins in plasma are unchanged, although the albumin level may be low and the globulin fraction may be elevated. The increase in the concentration of gamma globulins is caused by the development of antibodies. The level of potassium in serum may also be increased as a result of RBC lysis.

Diagnosis. Frequently, for a number of reasons, organism recovery and subsequent identification are more difficult than the textbooks imply. It is very important that this fact be recognized, particularly when one is dealing with a possibly fatal infection with *P. falciparum*. When requests for malarial smears are received in the laboratory, some patient history information should be made available to the laboratorian. This information should include the following:

1. Where has the patient been, and what was the date of return to the United States? ("Where do you live?")
2. Has malaria ever been diagnosed in the patient before? If so, which species was identified?
3. What medication (prophylaxis or otherwise) has the patient received, and how often? When was the last dose taken?
4. Has the patient ever received a blood transfusion? Is there a possibility of other needle transmission (drug user)?
5. When was the blood specimen drawn, and was the patient symptomatic at the time? Is there any evidence of a fever periodicity? Answers to such questions may help eliminate the possibility of infection with *P. falciparum*, usually the only species other than *Plasmodium knowlesi* that can rapidly lead to death.

Often the diagnosis of malaria is considered, and a single blood specimen is submitted to the laboratory for examination; however, single films or specimens cannot be relied on to exclude the diagnosis, especially when incomplete prophylactic medication or therapy is used. Incomplete use of antimalarial agents may be responsible for reducing the numbers of organisms in the peripheral blood, thus leading to a blood smear that contains few organisms, which then reflects a low parasitemia when in fact serious disease is present. Patients with a relapse case or an early primary case may also have few organisms in the blood smear (Table 12.1).

It is recommended that both thick and thin blood films be prepared *immediately* on admission of the patient, and at least 200 to 300 oil immersion fields should be examined on both films before a negative report is issued. Since one

TABLE 12.1 Parasitemia determined from conventional light microscopy: clinical correlation[a]

% Parasitemia	No. of parasites/µl	Clinical correlation[b]
0.0001–0.0004	5–20	Number of organisms that are required for a positive thick film (sensitivity) **Note** Examination of 100 TBF fields (0.25 µl) may miss infections up to 20% (sensitivity of 80–90%); at least 300 TBF fields should be examined before reporting a negative result. **Note** Examination of 100 THBF fields (0.005 µl); at least 300 THBF should be examined before reporting a negative result. Both TBF and THBF should be examined for every specimen submitted for a suspected malaria case. *One set (TBF + THBF) of negative blood films does not rule out a malaria infection.*
0.002	100	Patients may be symptomatic below this level, particularly if they are immunologically naive (no prior exposure to malaria: travelers).
0.2	10,000	Level above which immune patients will exhibit symptoms. Level can be seen in travelers (immunologically naive); however, results may also be *much* lower in this type of patient (traveler).
0.1	5,000	BinaxNOW rapid lateral flow method (dipstick) (>5,000 = 99.7% sensitivity for *P. falciparum*). BinaxNOW rapid lateral flow method (dipstick) (>5,000 = 93.5% sensitivity for *P. vivax*). Below this level of parasitemia, the test sensitivity declines. A negative test does NOT RULE OUT MALARIA.[c]
2	100,000	Maximum parasitemia of *P. vivax* and *Plasmodium ovale* (infect young RBCs only)
2–5	100,000–250,000	Hyperparasitemia, severe malaria,[d] increased mortality
10	500,000	Exchange transfusion may be considered; high mortality

[a] Adapted from references 3 and 5.

[b] TBF, thick blood film; THBF, thin blood film.

[c] The BinaxNOW Malaria Test (Inverness Medical, Scarborough, ME) is FDA approved. This test detects antigen from both viable and nonviable malaria organisms, including gametocytes and sequestered *P. falciparum* parasites. Test performance depends on antigen load in the specimen and may not directly correlate with microscopy performed on the same specimen. Samples with positive rheumatoid factor titers may produce false-positive results. Analytical reactivity testing demonstrates that the pan-malarial test line on the BinaxNOW test is capable of detecting all four malaria species. However, during clinical trials, insufficient data were generated to support clinical performance claims for the detection of *P. malariae* or *P. ovale*. Although this test was developed without considering the fifth human malaria, *Plasmodium knowlesi*, cases have been documented to be positive using the BinaxNOW. However, use of this test for the detection of *P. knowlesi* should not be considered acceptable. Modification of the test to include *P. knowlesi* is under way. Clinical performance claims for this test are made for *P. falciparum* and *P. vivax* detection only. The test is not intended for use in screening asymptomatic populations. IF THIS TEST IS NEGATIVE, STAT THICK AND THIN BLOOD FILMS MUST BE EXAMINED IMMEDIATELY (BinaxNOW Malaria Test package insert). Both the BinaxNOW Positive Malaria Control and the BinaxNOW Malaria Test are available commercially (Inverness Medical, Scarborough, ME).

[d] World Health Organization criteria for severe malaria are parasitemia of >10,000/µl and severe anemia (hemoglobin <5 g/liter). Prognosis is poor if >20% of parasites are pigment-containing trophozoites and schizonts and/or if >5% of neutrophils contain visible pigment.

set of negative films does not rule out malaria, additional blood specimens should be examined over a 36-h time frame. Although Giemsa stain is recommended for all parasitic blood work, the organisms can also be seen with other blood stains such as Wright's stain or the rapid stains. Blood collected with EDTA anticoagulant is acceptable; however, if the blood remains in the tube for any length of time, true stippling may not be visible within the infected RBCs (*P. vivax*, as an example). Also, when using anticoagulants, it is important to remember that use of the proper ratio between blood and anticoagulant is necessary for good organism morphology. If the blood stands for >2 h prior to blood film preparation, organism distortion is very likely, with the morphology beginning to mimic that seen with *P. malariae*. If the blood stands for >6 h prior to blood film preparation, the organisms will disintegrate.

Malaria is one of the few parasitic infections considered to be immediately life-threatening, and a diagnosis of *P. falciparum* malaria should be considered a medical emergency because the disease can be rapidly fatal. Any laboratory providing the expertise to identify malarial parasites should do so on a 24-h basis, 7 days/week.

Characteristics of *P. falciparum* (malignant tertian malaria) include the following:

1. 36- to 48-h cycle
2. Tends to infect any cell regardless of age; thus, very heavy infection may result
3. All sizes of RBCs
4. No Schüffner's dots (Maurer's dots: may be larger, single dots, bluish)
5. Multiple rings per cell (only young rings, gametocytes, and occasional mature schizonts are seen in peripheral blood)
6. Delicate rings; may have two dots of chromatin per ring, appliqué or accolé forms
7. Crescent-shaped gametocytes

Note Without the appliqué form, Schüffner's dots, multiple rings per cell, and other developing stages, differentiation among the species can be very difficult. It is obvious that the early rings of all five species can mimic one another very easily. Remember that one set of negative blood films cannot rule out a malaria infection, and this information must be conveyed to the physician.

Characteristics of *P. vivax* (benign tertian malaria) include the following:

1. 48-h cycle
2. Tends to infect young cells
3. Enlarged RBCs
4. Schüffner's dots after 8 to 10 h
5. Delicate ring
6. Very ameboid trophozoite
7. Mature schizont contains 12 to 24 merozoites

Reporting Parasitemia. It is important to report the level of parasitemia when blood films are reviewed and found to be positive for malaria parasites. Because of the potential for drug resistance in some of the *Plasmodium* species, particularly *P. falciparum*, it is important that every positive smear be assessed and the parasitemia reported using the exact same method on follow-up specimens as on the initial specimen. This allows the parasitemia to be monitored after therapy has been initiated. In cases where the patient is hospitalized, monitoring should be performed at 24, 48, and 72 h after initiation of therapy. Generally, the parasitemia will drop very quickly within the first 24 h; however, in cases of drug resistance, the level may appear to drop and then begin to rise again or the level may not decrease but actually increase over time.

Case 16

A 37-year-old private contractor returning from Afghanistan presented to his physician with a lesion on his arm. About 6 weeks earlier, he had noticed a small, red area on his arm. There was some itching, and over the next few weeks, the area ulcerated and measured about an inch in diameter. There was no drainage. He indicated that he was feeling well and had not had any fever, chills, anorexia, or weight loss. His medical history was unremarkable. The ulcer had a moist base and raised borders as seen in Fig. 12.22 (22, 23).

Questions

1. Based on the patient's history, what presumptive diagnosis might be appropriate?
2. What other diagnostic tests might be suggested at this point?
3. Based on additional diagnostic testing, what would you expect to find in order to support the presumptive diagnosis?
4. Should any special precautions be taken when processing and examining the clinical material? Why or why not?

Correct Identification

The lesion in Fig. 12.22 could have been caused by a number of agents; however, some options became much more likely after the patient's travel and work history were reviewed. The patient had traveled and worked in Iraq and Afghanistan. He did not remember being bitten at any specific time, but he did indicate that there were lots of flies (sand flies) present. The images shown in Fig. 12.23 and 12.24 were obtained from a skin biopsy specimen that was examined in pathology, and the organisms were isolated in culture (Fig. 12.23 and 12.24). This patient had cutaneous leishmaniasis.

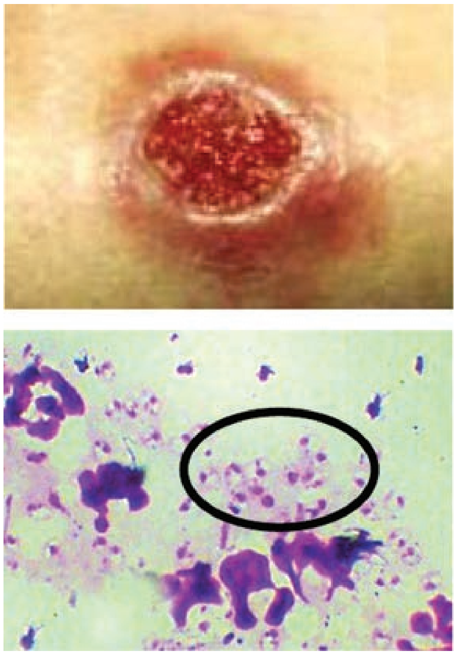


Figure 12.22 Case 16. (Upper) Lesion on patient's arm; (lower) skin biopsy specimen from margin of lesion.
doi:10.1128/9781555819002.ch12.f22

It is important to remember that with cutaneous and/or mucocutaneous lesions, the only area where the parasites are generally found is the lesion itself. Unless the infection is the visceral type (*Leishmania donovani*), no organisms are found in the spleen, bone marrow, and/or liver. There may be exceptions in cutaneous or mucocutaneous types of disease in which the amastigotes are found in other parts of the reticuloendothelial system; however, this tends to be unusual.

Leishmania major, *L. tropica*, *L. aethiopica*, and, rarely, *L. infantum* cause cutaneous disease in the Old World; disease manifestations include nodular and ulcerative skin lesions. Local geographic names for cutaneous leishmaniasis include Oriental sore, Baghdad boil, Delhi boil, Biskra button, and Aleppo evil.

The lesions of Old World cutaneous leishmaniasis are very similar to those seen with New World cutaneous disease. Weeks to months after infection, an erythematous, often pruritic papule develops at the bite site. This

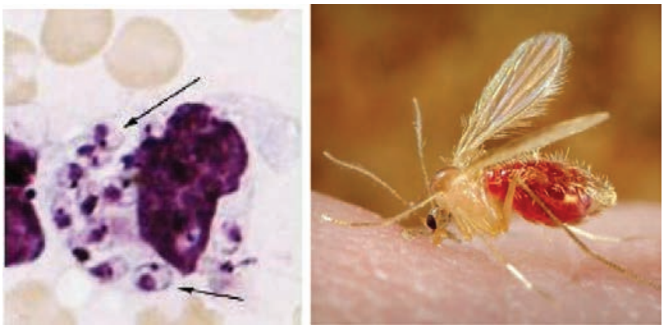


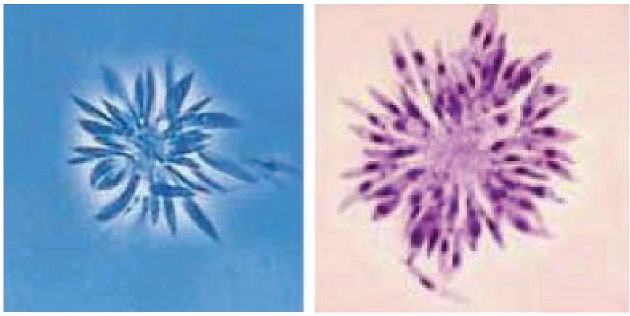
Figure 12.23 Case 16. Cutaneous leishmaniasis. (Left) Skin macrophage containing Leishman-Donovan bodies (note the nucleus and bar-shaped kinetoplast, arrows); (right) sand fly vector of leishmaniasis. (Images courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch12.f23

papule may become scaly and enlarged, developing a central ulcer surrounded by a raised margin. Disease progression at this point will vary, depending on the species involved. Lesions may be single or multiple and usually occur on exposed areas of the body such as the face, hands, feet, arms, and legs. In general, all lesions on a patient have a similar appearance and progress at the same speed.

The original lesion may remain as a flattened plaque or may progress, with the surface becoming covered with fine, papery scales. These scales are dry at first but later become moist and adherent, covering a shallow ulcer. As the ulcer enlarges, it produces an exudate and may develop a crust. The edge of the lesion is usually raised. Depending on the species, satellite lesions are common and merge with the original lesion.

In areas of the world where physicians are very familiar with leishmaniasis, the diagnosis may be made on clinical grounds. However, in other areas of the world, where the disease is rare, the condition may not be recognized as leishmaniasis. Definitive diagnosis depends on demonstrating the amastigotes in tissue specimens or the promastigotes in culture. Newer results suggest that PCR is a valuable tool for the diagnosis of leishmaniasis on a routine basis and can provide valuable epidemiologic information in areas of endemic infection. Cutaneous leishmaniasis may have to be differentiated from a number of other lesions and diseases, including basal cell carcinoma, tuberculosis, various mycoses, cheloid, and lepromatous leprosy.

Figure 12.24 Case 16. (Left) Wet mount of organisms from culture; (right) Giemsa-stained smear of culture sediment. doi:10.1128/9781555819002.ch12.f24



The ability to detect parasites in aspirates, scrapings, or biopsy specimens depends on the number of amastigotes present, the level of the host immune response, the absence or presence of bacterial and/or fungal contamination within the lesion, and whether the specimen is collected from an active or healing lesion. If the patient has multiple lesions, specimens should be collected from the more recent or active lesions. These lesions should be thoroughly cleaned with 70% alcohol, and necrotic debris should be removed to prevent the risk of bacterial and/or fungal contamination of the specimen. Also, the specimen should be taken from the advancing margin of the lesion; the central portion of the ulcer contains nothing but necrotic debris.

Appropriate specimens would include scrapings of the lesions, as well as a collection of several punch biopsy specimens, taken from the most active lesion areas. However, fine-needle aspiration cytology has also been recommended. Biopsy specimens can be divided and used for cultures and touch preparations; some material should always be saved and submitted for routine histologic examination. The biopsy specimen can be used to make impression smears (touch preparations); these smears should be prepared after portions of the specimen have been placed in culture media by using sterile techniques. After cleaning off any excess blood, a horizontal cut is made through the core of the specimen and the cut surface is gently touched to glass slides; multiple slides should be prepared. Once material has been set up for culture and touch preparations have been made, the remainder of the tissue can be sent to the pathology department for routine processing.

Culture media that have been used for the recovery and growth of the leishmaniae include Novy-MacNeal-Nicolle medium and Schneider's Drosophila medium supplemented with 30% fetal bovine serum. Patient cultures should not be set unless the laboratory maintains specific organism strains for quality control checks. Both the control and patient cultures should be examined twice weekly for the first 2 weeks and once a week thereafter for up to 4 weeks before they are called negative. Promastigote stages can be detected microscopically in wet mounts taken from centrifuged culture fluid. This material can also be stained with Giemsa stain or other blood stains to facilitate observation at a higher magnification.

A sensitive microcapillary culture method (MCM) was developed for the rapid diagnosis of cutaneous leishmaniasis (24). The MCM is superior to the traditional culture method (TCM) as determined by the smaller inoculum size, the higher sensitivity for detection of promastigotes, and the shorter time to the emergence of promastigotes. With lesion amastigote loads from grade III to 0, the positive rates and the periods for promastigote emergence were 3- to 4-fold higher and faster with the MCM than with the TCM, e.g., 83 to 97% positive in 4 to 7 days versus 20 to 40% positive in 15 to 30 days ($P = 0.0001$). This MCM has the advantage of simplicity and may be suitable for diagnostic use and for parasite retrieval in many other areas of endemic infection where parasites are known to be difficult to grow.

Key Points for Laboratory Identification

1. For a complete laboratory diagnosis, microscopic examination of stained touch preparations and cultures is recommended.
2. Aspirates or biopsy specimens should be taken from the margin or base of the most active lesion (papule or ulcer). The lesion should be cleaned before the sample is collected, to reduce the chances of contamination with fungi or bacteria.
3. Multiple slides should be prepared for examination.
4. Tissue imprints or smears should be stained with Giemsa stain (or one of the stains commonly used for blood smears) (see chapter 7).
5. Amastigote stages should be found within macrophages or close to disrupted cells.
6. If cultures are to be performed, specimens must be taken aseptically and control organism cultures should be set up at the same time (see chapter 8).
7. Cultures should be checked weekly for 4 weeks before they are declared negative.

Most Old World cutaneous leishmaniasis lesions are self-healing over a period of a few months to several years. This healing process confers immunity to individuals living within the immediate area of endemic infection; therefore, treatment is not recommended unless disfiguring scarring is a possibility. Local care of the lesion and treatment of secondary bacterial infection are essential for healing. Antileishmanial therapy is indicated in immunocompromised hosts and patients with progressive, multiple, or critically located lesions. Pentavalent antimony compounds remain the main therapeutic option for all species. They are given intravenously, intramuscularly, or intralesionally. Cryotherapy and some systemic antifungal agents have been used successfully. Oral azoles are promising new treatments for lesions caused by *L. major*.

Case 17

A 61-year-old man presented to his physician with a history of symptoms, beginning about 3 weeks after he returned to southern Texas from a photographic safari to Africa; he had visited Kenya, Tanzania, Uganda, and South Africa. Symptoms included general malaise followed by fever, chills, headaches, and back pain. He had seen a physician before returning to the United States and had received some antimalarial medication. He was an active wildlife photographer and was often exposed to a number of life zones and resident plants, animals, insects, etc., both at home and on trips outside of the United States.

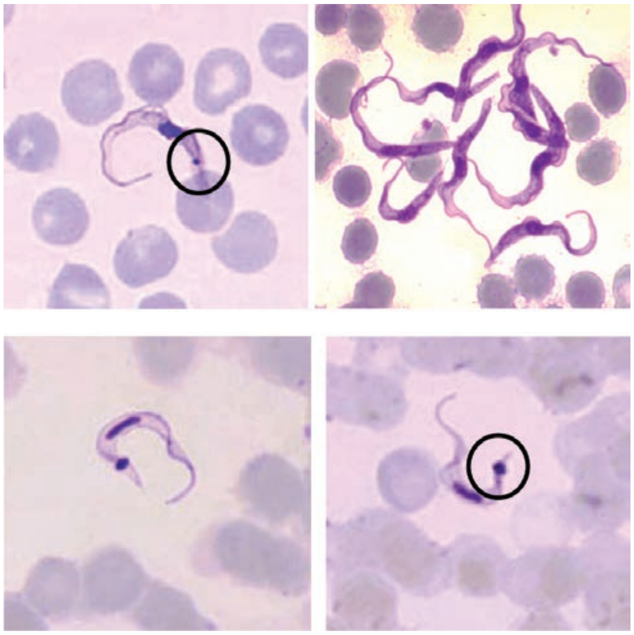
Although both the patient and several others in the group remembered being bitten by insects, he was the only one who was symptomatic. On examination, he had several swollen areas, including a lymph node on his neck and a draining lesion on his upper arm.

Blood was drawn for routine hematology procedures, and a specimen was also drawn to be sent to the microbiology laboratory for examination as blood films stained with Giemsa stain (or one of the other blood stains) (Fig. 12.25) (1–6). Based on his travel history, additional laboratory work revealed a slightly elevated white blood cell count with a relative lymphocytosis. His gamma globulin level was elevated, with a marked elevation of the immunoglobulin M (IgM) level.

Questions

1. In Fig. 12.25, what are the major differences between the two parasites seen (upper row and bottom row)?

Figure 12.25 Case 17. (Upper row, left) Objects seen in Giemsa-stained thin blood film; (right) example of organisms multiplying within the blood/patient blood film. (Bottom, left and right), Organisms in human blood films. Note the key structures indicated by circles. doi:10.1128/9781555819002.ch12.f25



2. Is there anything in the patient's history that would help you with the diagnosis? What and why?
3. How do you explain the marked elevation in the IgM level?
4. Is there anything in the life cycle that would explain the laboratory findings?

Correct Identification

The patient was suffering from African trypanosomiasis, probably caused by *Trypanosoma brucei rhodesiense* (East African trypanosomiasis) based on travel history (Kenya and Uganda), the severity of symptoms, and the overall description of the case. Although the man lived in southern Texas, where *Trypanosoma cruzi* (the cause of Chagas' disease, or American trypanosomiasis) is found, the African trypomastigotes and the American trypomastigotes look quite different (25, 26).

The African trypomastigotes (both East and West African trypomastigotes) (Fig. 12.25, upper row) have a very small kinetoplast (the dot at one end of the organism), while *T. cruzi* tends to have a very large kinetoplast that appears to go beyond the body of the organism (Fig. 12.25, bottom row). As both organisms can actually be found in a C-shape, this finding alone does not indicate infection with *T. cruzi*.

The course of infection in African trypanosomiasis occurs in three stages. Soon after inoculation of the metacyclic trypanosomes by an infected tsetse fly, an inflammatory lesion, called the trypanosomal chancre, develops at the site of inoculation. Trypomastigotes then invade the local lymphatics and later the bloodstream. After a time, the organisms invade the choroid plexus and enter the brain and CSF. There is quite a bit of variation between East African and West African trypanosomiasis. In the East African disease (caused by *T. brucei rhodesiense*), chancres are common and the hemolymphatic stage is severe, rapidly progressing to a fatal meningoencephalitis, often within months of infection. With the West African form of the disease (caused *by T. brucei gambiense*), chancres are uncommon, the hemolymphatic stage may even be undetected, and meningoencephalitis progresses very slowly, often over several years: hence the "sleeping sickness" syndrome.

During the course of the infection, the number of trypomastigotes in the blood fluctuates. This relapsing parasitemia is due to the host immune response to the parasites. Each decline in parasite number results from the antibody-mediated destruction of trypomastigotes bearing a particular variant surface glycoprotein (VSG). Each new wave of parasitemia represents the growth of a trypomastigote population expressing an antigenically different VSG. This process of antigenic variation is a feature of all African trypomastigotes. Since each trypomastigote expresses only one VSG at a time and has as many as 1,000 different VSG genes, the number of different variable antigen types that can be expressed during an infection is quite large. The continued response of the host to the new/different variable antigen types leads to *elevated gamma globulin levels, particularly the IgM levels*, including in the CSF. Generally, an individual who does not have an elevated IgM level almost certainly does not have African trypanosomiasis.

Key Points for Laboratory Identification

1. Trypomastigotes are highly infectious, and health care workers must use blood-borne pathogen precautions.
2. Trypomastigotes may be detected in aspirates of the chancre and enlarged lymph nodes in addition to blood and CSF.
3. Concentration techniques, such as centrifugation of CSF and blood, should be used in addition to thin and thick smears (Giemsa, Wright's, or rapid stains).
4. Trypomastigotes are present in largest numbers in the blood during febrile periods.
5. Multiple daily blood examinations may be necessary to detect the parasite.
6. Blood and CSF specimens should be examined during therapy and 1 to 2 months posttherapy.

All drugs currently used in the therapy of African trypanosomiasis are toxic and require prolonged administration. Treatment should be started as soon as possible and is based on the patient's symptoms and laboratory findings. The choice of antiparasitic drug depends on whether the CNS is infected. Specific details can be found in chapters 20 and 36.

Case 18

The patient was a 9-month-old male infant living in the United States, with multiple medical problems, who was admitted to the hospital with the complaint of irregular recurrent fever of 3 months' duration. He had received several blood transfusions over a period of several months, primarily for low hemoglobin levels. He became feverish with a temperature of 102 to 104°F, which did not respond to antimicrobials. His fever appeared to peak about every 4 days. Just prior to admission, the fever began to occur daily. On examination of both thick and thin blood films prepared from blood collected in EDTA tube (purple top), the images shown in Fig. 12.26 were seen. Both the thick and thin blood films were stained using Giemsa stain (1–6, 27).

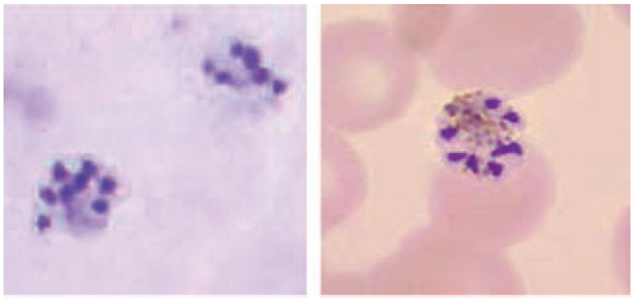


Figure 12.26 Case 18. Blood films stained with Giemsa stain. (Left) Thick blood film; (right) thin blood film. doi:10.1128/9781555819002.ch12.f26

Questions

1. Based on the patient's history, what parasitic infections might be suspected? Why?
2. Considering the organism morphology, is there enough detail to allow identification to the genus and species levels?
3. What differences might be seen if the blood films had been stained with Wright-Giemsa or rapid stains? Would this approach have been acceptable? Why or why not?
4. Would the request for blood films be considered a STAT request? Why or why not?

Correct Identification

The images (Fig. 12.26) obtained after the first blood specimen was drawn represent *Plasmodium malariae* developing and mature schizonts in a thick blood film (left) and a mature schizont (containing merozoites) in the thin blood film (right). Note the small size of the infected RBC (typical of *P. malariae*). The number of merozoites in the mature schizont is always a clue to the species identification; in this case there appear to be about eight merozoites.

The images obtained after the second blood draw (Fig. 12.27) represent developing trophozoites, which are spread across the infected RBC as "band" forms (again *P. malariae*). This configuration is not always seen but is very suggestive of *P. malariae*.

Key Characteristics of *P. malariae*

1. There is a 72-h cycle.
2. The parasite tends to infect old cells.
3. It infects small to normal-sized RBCs.
4. No Schüffner's dots are present; small dots (Ziemann's dots) may be present, but they are not considered true stippling.
5. There is a thick ring and a heavy chromatin dot.
6. "Band" form trophozoites are often present.
7. The mature schizont contains about eight merozoites, often arranged around the remaining clump of malarial pigment. This configuration of the merozoites is often called the "daisy" form.

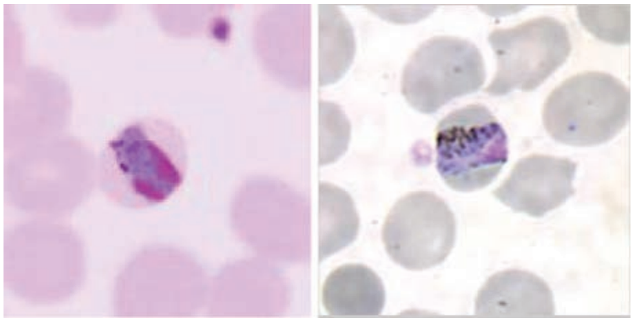


Figure 12.27 Case 18. Thin blood films stained with blood stains. *Plasmodium malariae* "band" forms are evident where the developing trophozoites spread across the RBC. Note that the infected RBCs are normal to smaller than normal size compared with the uninfected RBCs. Also, note the dark malarial pigment seen in the band form on the right. doi:10.1128/9781555819002.ch12.f27

Since this patient had received several blood transfusions, it is logical to assume that one of the transfusions was the source of the infection. Individuals can harbor *P. malariae* in the blood for many years at very low levels of parasitemia; these individuals are asymptomatic. Often, even with a complete blood donor history, the possibility of malaria infection with this organism is not suspected. Individuals have been known to harbor this parasite for up to 42 years. In spite of the patient's severe splenomegaly and splenectomy, he was considered cured after therapy with chloroquine and remained asymptomatic.

It is very important to realize that a single set of thick and thin blood films can be negative, even though the patient may be infected. In this case, both thick and thin blood films were positive. A second draw was taken to examine the thick and thin blood films for additional stages and/or evidence of a mixed infection. Venipunctures were performed for both blood draws, with the recommended EDTA anticoagulant in the lavender (purple)-top tube. It is important that the slides be prepared as quickly as possible after the blood draw to prevent the organism distortion and possible loss that can occur if the blood is allowed to stand for several hours prior to slide preparation. Remember, every request for malaria blood films should always be considered a STAT request and the laboratory coverage should be 24 h/day, 7 days/week.

Examination of the thin blood film is relatively simple when the parasitemia is high, as in Fig. 12.27. However, a returning traveler with his or her first malaria infection may experience the typical clinical symptoms of high fever, chills, myalgia, and headache with a much lower parasitemia. Also, these patients may present to the emergency room with vague symptoms that do not represent the typical textbook description; they may have malaise, a steady low-grade fever, and even diarrhea. These low levels of parasitemia are often impossible to detect using thin blood film examination only. For this reason, the key to successful detection of malaria parasites in the peripheral blood is the examination of thick and thin blood films from every patient suspected of having malaria (or any patient from whom blood is submitted to the laboratory for blood film examination).

Thick films allow a larger amount of blood to be examined, which increases the possibility of detecting light infections. Species identification from the thick-film examination, particularly in the case of malaria, may be difficult for workers with little experience examining thick blood films. The morphologic characteristics of blood parasites are best seen in thin films, particularly the relationship between the sizes of infected and uninfected RBCs. However, in cases with a low parasitemia, the identification to the species level may have to be accomplished by thick-film examination.

The accurate examination of thick and thin blood films and identification of parasites depend on the use of absolutely clean, grease-free slides for preparation of all blood films. Old (unscratched) slides should be cleaned first in detergent and then with 70% ethyl alcohol; new slides should also be cleaned with alcohol before use.

Blood films are usually prepared when the patient is admitted; in instances in which malaria is a possible diagnosis, samples should be taken at intervals of 6 to 8 h for at least three successive days after the first set of negative smears, particularly if *P. falciparum* has not been excluded as a diagnosis. Extensive therapy information is available in chapter 36.

Case 19

A 62-year-old female, currently living in New Mexico, was admitted to the hospital with a 3-week history of fever, headache, and overall malaise. She experienced occasional chills, which appeared to coincide with the fevers. Her past medical history appeared to be unremarkable, with the exception of an emergency appendectomy about 20 years earlier. Routine blood examinations for parasites revealed the images shown in Fig. 12.28.

On the basis of this blood film, the patient was diagnosed with malaria. With this diagnosis, a more comprehensive history was taken in order to determine how and when she had acquired this *Plasmodium* sp. infection. Based on the diagnosis of malaria, what other information would you like to have in order to better explain her illness within the context of her medical history? Do your questions match any of those presented below?

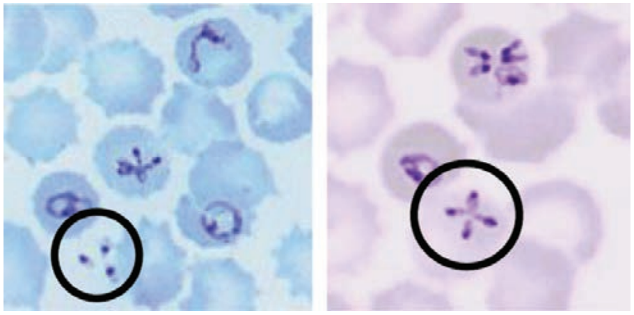


Figure 12.28 Case 19. Thin blood films stained with blood stains. In the image on the left, note the three small rings that are seen outside of the RBC (circle); this is very unusual for a case of malaria, but can be seen in blood films in which *Babesia* spp. are present. There are a number of rings in the image on the right, including the typical Maltese cross formation with four ring forms (circle). doi:10.1128/9781555819002.ch12.f28

Questions

1. In Fig. 12.28, do the structures within the circles support or invalidate the diagnosis of *Plasmodium*?
2. Is there anything in the patient's history that would support the diagnosis? Yes or No?
3. Based on the history, what are the potential routes of infection?
4. Is there anything in the life cycle that would explain the laboratory findings?
5. What other organisms might mimic *Plasmodium*?

Correct Identification

Since the patient had no significant travel history outside of the United States, no history of blood transfusion or shared needle related to possible drug abuse, it is highly unlikely that this patient had malaria. The one question that was not asked relates to her place of residence, possibly by an airport, or whether she worked close to an airport. There is an unusual situation in which infected mosquitos may be transported from one country to another through baggage or the interior of airplanes. Once the plane lands, the mosquitos are free to disseminate throughout the new territory. Very rarely, someone who lives by or works at an airport might be bitten by an infected mosquito, thus transmitting a malaria infection within an area where malaria is not endemic. Continuous endemic malaria within the United States ended in the 1940s. This has been termed "airport" malaria. A patient with absolutely no relevant history pertaining to a malaria infection could still acquire the infection through proximity to an airport. Again, this is a rare situation, but it has been documented a number of times.

On further questioning, the woman had visited her niece who lived on Long Island for several weeks. Both had dogs, frequently requiring tick removal after running in the woods. Although she did not recall being bitten by ticks, there was considerable tick contact on a daily basis. Based on this additional information, it was likely that the correct diagnosis was babesiosis. The patient was treated and recovered with no recurrence of her illness. *Babesia* infections described in California and in other parts of the world are quite different from those seen in the northeastern United States (*Babesia microti*), where the infection is most often subclinical. Infections in the western United States (*B. duncani*, others not yet given species names) and Europe (*B. divergens*) tend to present as a fulminant, febrile, hemolytic disease affecting splenectomized or immunosuppressed individuals. Additional information can be found in chapter 18 (28).

This case represents a situation where the clinical history was limited; many of the appropriate questions that should have been asked were not considered until the diagnosis of malaria failed to match the additional questions that were asked during some follow-up discussions with the patient.

When requests for malarial smears are received in the laboratory, some patient history information should be made available to the laboratorian. This information should include the following questions, particularly if the patient has a relevant travel history:

1. Where has the patient been, and what was the date of return to the United States? Where does the patient live?
2. Has malaria ever been diagnosed in the patient before? If so, what species was identified (possibility of a relapse infection)?
3. What medication (prophylaxis or otherwise) has the patient received, and how often? When was the last dose taken?
4. Has the patient ever received a blood transfusion? Is there a possibility of other needle transmission (drug abuse)?
5. When was the blood specimen drawn, and was the patient symptomatic at the time? Is there any evidence of a fever periodicity?

If the patient does not have a relevant travel history, then additional questions must be addressed, particularly if the patient has a positive blood film with organisms that resemble *Plasmodium* spp.

1. Does she have a history of travel to Long Island, especially Montauk Point; Hawaii, Alaska, and Florida; Nantucket Island, Martha's Vineyard, and Shelter Island or nearby areas; California, Washington, Georgia? What about travel to Europe? These are areas where human babesiosis has been acquired via tick bites.
2. Has the patient been splenectomized? Based on her medical history, this was not the case. However, in splenectomized individuals, severe illness can occur, leading to death.

Comments on the Images (Fig. 12.28)

The *Babesia* ring forms mimic those of *Plasmodium falciparum*, thus the mistaken diagnosis of "malaria." It is important to review additional slides for some comparative differences and comments. In the image on the left, note the three small rings that are seen outside of the RBC (circle); this is very unusual for a case of malaria, but can be seen in blood films in which *Babesia* spp. are present. There are a number of rings in the image on the right, including the typical "Maltese cross" formation with four ring forms (circle). Although this ring formation tends to be diagnostic for *Babesia* infections, it is not always seen.

Key Points for Laboratory Identification

1. Blood films should be prepared immediately upon admission of the patient. Travel history and information on possible exposure to tick-infested area, recent blood transfusion, or splenectomy are very important.
2. Both thick and thin blood films should be prepared and examined. At least 200 to 300 oil immersion fields should be examined before the smears are considered negative.
3. Wright's, Wright-Giemsa, Giemsa, or the rapid stains can be used; if the WBCs look acceptable, then any parasites will also be acceptable (morphology, color).
4. *Babesia* parasites may be missed by automated differential instruments. Even with technologist review of the smears, a light parasitemia is very likely to be missed.
5. There does not have to be any significant history of travel outside the United States. If organisms are seen, they will mimic *P. falciparum* rings.
6. It is important to report the organism parasitemia, just as you would with a case of malaria.

Case 20

The patient was a 44-year-old male living within the United States. He presented to the clinic with a 10-day history of irregular recurrent fever, shivering, nausea, and anorexia. Several weeks before, he had returned from an extended business trip to Thailand and India. On examination of both thick and thin blood films prepared from blood collected in EDTA tubes (purple top), the images shown in Fig. 12.29 were seen. Please comment on the identification of the organisms seen.

Questions

1. In Fig. 12.29, do these organisms support or invalidate the diagnosis of *Plasmodium*?
2. Is there anything in the patient's history that would support the diagnosis? Yes or No?
3. Based on the history, what are the possible *Plasmodium* species that could be involved?
4. What is there about the travel history that could be significant in terms of the species identification?

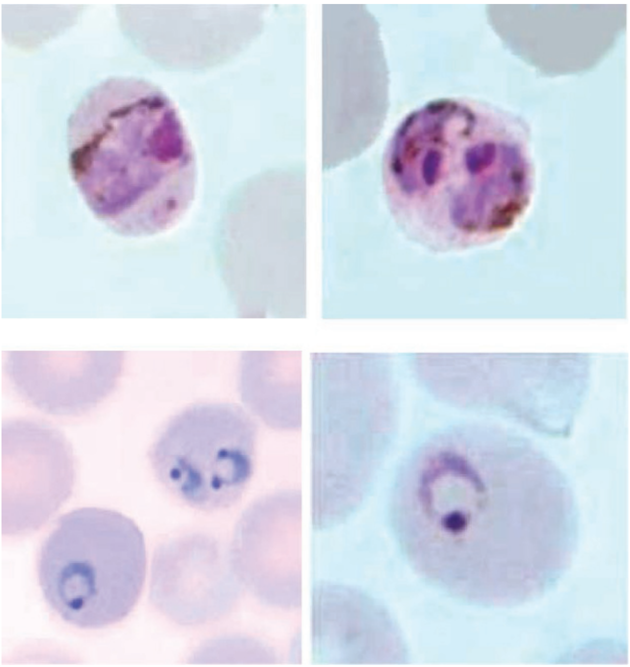
The images presented after the first blood specimen was drawn resemble a *P. malariae* band form (Fig. 12.29, upper row, left). Note the small size of the infected RBC (typical of *P. malariae*). The image on the right demonstrates a developing schizont that also looks like *P. malariae*. The ring forms (bottom row) are characteristic of *P. falciparum*.

Additional Patient and Test Information

The patient had a 0.8% parasitemia that appeared to be a mixed infection with *P. falciparum* (multiple rings/RBC) and *P. malariae* (band form trophozoites). Rapid diagnostic tests were performed (BinaxNOW test; Inverness Medical, Sevres, France) with the following results.

The kit was positive for the Pan-Plasmodium (aldolase common to the four species of human malaria) line (often interpreted as *P. vivax*); however, the histidine-rich protein 2 (HRP2), a specific antigen of *P. falciparum*, was negative.

Figure 12.29 Case 20. Thin blood films stained with blood stains (courtesy of the CDC Public Health Image Library). These organisms tend to mimic *Plasmodium falciparum* and *P. malariae*. doi:10.1128/9781555819002.ch12.f29



Given the discrepancy between the thin blood films and the rapid test results, another laboratory was asked to confirm the results. Their results with the rapid test were identical (positive Pan-Plasmodium line, negative *P. falciparum* line). Although PCR was used and only the *P. vivax* PCR was positive, the parasite morphology on the blood films was not compatible with *P. vivax*.

Since this patient had spent time in Southeast Asia, followup sequencing was done to confirm the malarial species. A basic local alignment search tool (BLAST) search of the GeneBank database with the sequence obtained (1,000 bp) showed 100% identity with *P. knowlesi* sequences (29, 30).

Key Characteristics of *P. knowlesi*

1. 24-hour cycle
2. Tends to infect any cell regardless of age, thus very heavy infection may result
3. All sizes of RBCs, but most tend to be normal size
4. No Schüffner's dots (faint, clumpy dots later in cycle)
5. Multiple rings/cell (may have two or three)
6. Delicate rings, may have two or three dots of chromatin/ring, appliqué forms
7. Band form trophozoites commonly seen
8. Mature schizont contains 16 merozoites, no rosettes
9. Gametocytes round, tend to fill the cell
10. The early stages tend to mimic *P. falciparum*, while the later stages mimic *P. malariae*; mixed infections of the two have also been reported incorrectly.

A relatively large focus of human infections with the simian malaria, *P. knowlesi*, has been reported in Malaysian Borneo; cases have also been reported from Thailand, Myanmar, China, Philippines, and Singapore. The early blood stages of *P. knowlesi* resemble those of *P. falciparum*, whereas the mature blood stages and gametocytes resemble those of *P. malariae*. Unfortunately, these infections are often misdiagnosed as the relatively benign *P. malariae*; infections with *P. knowlesi* can be fatal, particularly considering the life cycle time of 24 h (the fastest of all the human malarias). *P. knowlesi* infection should be considered in patients with a travel history to forested areas of Southeast Asia, especially if *P. malariae* is diagnosed, unusual forms are seen with microscopy, or if a mixed infection with *P. falciparum*/*P. malariae* is diagnosed.

Because the disease is potentially fatal, proper identification to the species level is critical. Patients exhibit chills, minor headaches, and daily low-grade fever. Patients who present with *P. malariae* hyperparasitemia diagnosis by microscopy should receive intensive management as appropriate for severe *P. falciparum* malaria, assuming the infection is actually caused by *P. knowlesi*.

Comments on Diagnostic Methods

It is very important to realize that a single set of thick and thin blood films can be negative, although the patient may be positive. In this case, both thick and thin blood films were positive. A second draw was taken to examine the thick and thin blood films for additional stages and/or evidence of a mixed infection. Venipunctures were performed for both blood draws, with the recommended EDTA anticoagulant in the lavender (purple) top tube. It is important that the slides be prepared as quickly as possible after the blood draw, in order to prevent organism distortion and possible loss that can occur if the blood is allowed to stand for a period of hours prior to slide preparation. Remember, every request for malaria blood films should always be considered a STAT request and the laboratory coverage should be 24 hours/day, 7 days/week (ordering, collection, processing, examination, reporting/consultation).

STAT testing

The topic of STAT testing for both *P. malariae* and *P. knowlesi* is important to emphasize for both organisms. Examination of the thin blood film is relatively simple when the parasitemia is high, as in the slide shown in Fig. 12.29. However, a returning traveler (immunologically naive with no prior exposure to malaria) with his or her first malaria infection may experience the typical clinical symptoms of high fever, chills, myalgia, and headache with a much lower parasitemia. Also, these patients may present to the emergency room with vague symptoms that do not represent the typical textbook description; they may have malaise, a steady low-grade fever, and may even have diarrhea. These low levels of parasitemia are often impossible to detect using thin blood film examination only. For this reason, the key to successful detection of malaria parasites in the peripheral blood is the examination of thick and thin blood films from every patient suspected of having malaria (or any patient from whom blood is submitted to the laboratory for blood film examination).

Thick films allow a larger amount of blood to be examined, which increases the possibility of detecting light infections. Species identification from the thick film examination, particularly in the case of malaria, may be difficult for those with little experience examining thick blood films. The morphological characteristics of blood parasites are best seen in thin films, particularly the relationship between the size of the infected RBC and those that are uninfected. However, in cases with a low parasitemia, the identification to the species level may have to be accomplished by thick film examination.

The accurate examination of thick and thin blood films and identification of parasites depend on the use of absolutely clean, grease-free slides for preparation of all blood films. Old (unscratched) slides should be cleaned first in detergent and then with 70% ethyl alcohol; new slides should also be cleaned with alcohol before use.

Blood films are usually prepared when the patient is admitted; in instances in which malaria is a possible diagnosis, after the first set of negative smears, samples should be taken at intervals of 6 to 8 h for at least 3 successive days, particularly if *P. falciparum* or *P. knowlesi* has not been excluded as a diagnosis.

Key Points for Laboratory Identification

1. Blood films should be prepared immediately upon admission of the patient. Travel history and information on possible exposure to tick-infested area, recent blood transfusion, or splenectomy are very important.
2. Both thick and thin blood films should be prepared and examined. At least 200 to 300 oil immersion fields should be examined before the smears are considered negative.
3. Wright's, Wright-Giemsa, Giemsa, or the rapid stains can be used; if the WBCs look acceptable, then any parasites will also be acceptable (morphology, color).
4. Blood parasites may be missed by automated differential instruments. Even with technologist review of the smears, a light parasitemia is very likely to be missed.
5. Travel history information is critical, particularly for cases of possible *P. knowlesi* infection.
6. It is important to report the organism parasitemia, just as you would with all cases of malaria.

Diagnostic Methods and Proficiency Testing

Case 21

The patient was a 54-year-old man from the United States, who had received a liver transplant 14 months earlier. He had traveled throughout the world as a professional consultant (Europe, Asia, Central and South America, Australia, New Zealand, etc.). He was diagnosed as having diarrhea, cough, and general malaise. He had a history of vague health problems since the transplant but did not seek medical attention for several weeks. The first stool specimen was submitted to the laboratory. After examination of the concentration sediment and permanent stained smears from all three specimens, the objects shown in Fig. 12.30 were seen (1–6, 31–35).

The structure on the right measures approximately 16 µm, and the structures on the left measure approximately 65 µm. The structure on the right is stained with the standard Wheatley's modification of the Gomori trichrome, while the structure on the left is a wet preparation of the sedimentation concentration.

After submission of the other two stool specimens, it was also decided to perform special stains for the coccidia and microsporidia. The objects shown in Fig. 12.31 were seen on the stained smears; structures on the left measure ca. 4 to 6 µm, while those on the right measure ca. 1.5 µm.

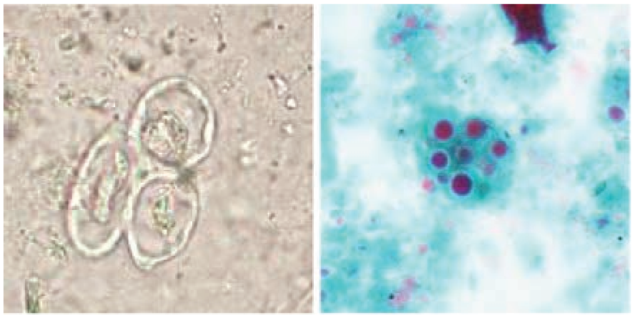
Questions

1. Based on Fig. 12.30, can you identify the objects?
2. Considering the patient's history, what types of parasites would you suspect? Why?
3. What additional laboratory tests might have been performed prior to the routine O&P examination? Do you think other laboratory tests would have been more appropriate? Why or why not?
4. Why should special stains or other testing options be considered for a patient with this type of history? What would you be looking for?

Correct Identification

The images presented after the first stool specimen represent artifact material (Fig. 12.30). The structure on the right mimics an *Entamoeba coli* cyst; however, this structure is not an organism but an artifact. The structure on the left tends to resemble helminth eggs, but they are vegetable cells and not parasites. Unfortunately, even when structures are within the correct size range, the morphology may not be correct or accurate enough to identify the structure as a true parasite.

Figure 12.30 Case 21. Objects seen in a wet mount (left) and a permanent stained smear (right). The structure on the right measures approximately 16 µm, and the structures on the left measure approximately 65 µm.
doi:10.1128/9781555819002.ch12.f30



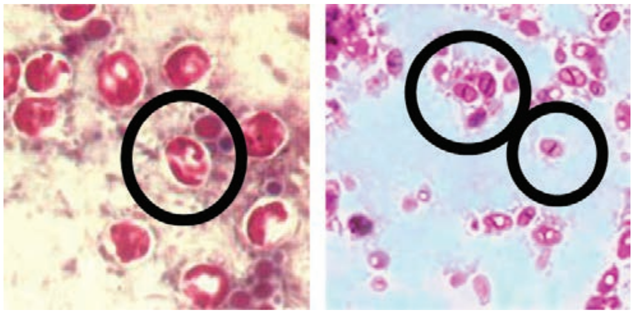


Figure 12.31 Case 21. Objects in a concentrated fecal sediment stained with modified acid-fast stain. (Left) Note the individual sporozoites within the oocyst wall (circle); these are not visible in every oocyst. Remember, all *Cryptosporidium* oocysts are immediately infectious when passed, regardless of whether the sporozoites are visible or are not seen within the oocyst. (Right) Note the cross/diagonal lines within the microsporidian spores (circles), representing the polar tubule.
doi:10.1128/9781555819002.ch12.f31

The two images obtained when special stains were performed provide excellent examples of *Cryptosporidium* spp. oocysts (Fig. 12.31, left) and microsporidial spores (Fig. 12.31, right), probably *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis*. In the left image (circle), note the individual sporozoites within the oocyst wall; these are not visible in every oocyst. Remember, all *Cryptosporidium* oocysts are immediately infectious when passed, regardless of the stool consistency. In the right image (circles), note the cross/diagonal lines within the microsporidian spores, representing the polar tubule.

Because this patient is a posttransplant (liver) patient, who has been and continues to be immunosuppressed, continuous diarrhea with negative O&P examinations should suggest the use of special stains for the coccidia (modified acid-fast stains) or microsporidia (modified trichrome stains). These diagnostic options are important, particularly if the patient is immunosuppressed and continues to be symptomatic with diarrhea.

Although there are immunoassays available for the diagnosis of cryptosporidiosis (enzyme immunoassay, fluorescent-antibody assay, cartridge formats), many laboratories continue to use the modified acid-fast stain. It is important to remember that the destaining (acid) step should be performed quickly and the acid content should not be too high. If the percentage is above 3% acid, too much color may be removed from the oocysts. Color loss can be a definite problem for the identification of *Cyclospora cayetanensis* (modified acid-fast variable) but is less of a problem with *Cryptosporidium* spp. Many laboratories are using this stain with a 1% acid destain step. Both *Cryptosporidium* and *Cyclospora* look fine when this approach is used. However, regardless of the destain step, some of the *C. cayeta-*

nensis oocysts may appear pale pink or even colorless. It is also recommended that these special stains (for both coccidia and microsporidia) be performed on concentration sediment to enhance sensitivity (centrifuge for 10 min at 500 × *g*).

Currently, there are no FDA-approved available immunoassays for detection of the microsporidian spores in clinical specimens. There are a number of different modified trichrome stain methods, some hot and some cold; they all work well, so selection of a particular method often depends on personal preferences among laboratory personnel. Again, it is important to perform these stains on concentrated fecal sediment. Remember that other clinical specimens can also be stained (sputum, urine, etc.) and may contain less debris than that seen in a fecal specimen.

Case 22

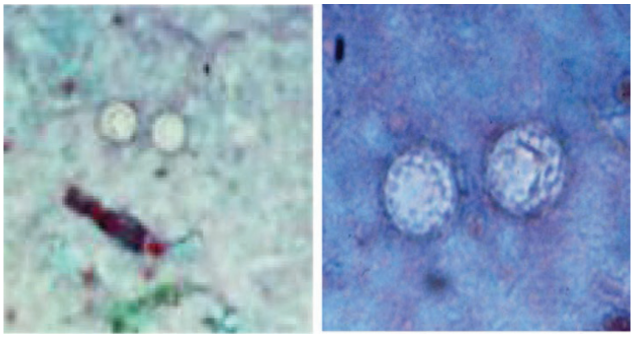
The patient was a 53-year-old male living in Australia, HIV negative, who was previously treated for tuberculosis several years before. He presented with weight loss, cough with purulent expectoration, and pulmonary fibrosis. Stains of sputum specimens were negative for acid-fast bacilli; however, a pink structure was seen that was unidentified. Because he complained of intermittent watery diarrhea, routine O&P examinations were performed. In a routine stool examination for ova and parasites, the image shown in Fig. 12.32 (two different stool specimens) was seen on the Wheatley trichrome stain.

Additional information obtained from the patient indicated he had just returned from a cruise about a month ago; routine testing for stool pathogens was negative. He mentioned that a friend who had taken the same cruise had also complained of diarrhea on his return.

Questions

1. Based on Fig. 12.31, can you identify the objects?
2. Considering the patient's history, what types of parasites would you suspect? Why?
3. What additional patient history might have been helpful? Do you think other laboratory tests would have been appropriate? Why or why not?
4. Why should special stains or other testing options be considered for a patient with this type of history? What would you be looking for?

Figure 12.32 Case 22. These images (trichrome stain) contain structures that did not stain using Wheatley's trichrome stain; they measure approximately 8 to 10 µm and appear as "ghost" cells. doi:10.1128/9781555819002.ch12.f32



Correct Identification

The images in Fig. 12.32 (trichrome stain) show *Cyclospora cayetanensis* oocysts; these oocysts measure approximately 8 to 10 µm and will not stain with the routine trichrome stain. They appear as "ghost" cells. Based on the structures seen in the routine trichrome stained slides, additional slides were prepared from concentrated stool sediment (500 × *g* for 10 min) and stained using the modified acid-fast stain with a 1% to 3% acid decolorizer (no stronger). Although the *Cyclospora* oocysts were stained with the Ziehl-Neelsen stain (patient history), it is also possible that the oocysts might not have stained due to the use of the stronger acid-alcohol decolorizer.

In *Cyclospora* infections, there is generally 1 day of malaise and low-grade fever, with rapid onset of diarrhea of up to seven stools per day. There may also be fatigue, anorexia, vomiting, myalgia, and weight loss with remission of self-limiting diarrhea in 3 to 4 days, followed by relapses lasting from 4 to 7 weeks. In patients with AIDS, symptoms may persist for as long as 12 weeks; biliary disease has also been reported in this group. Diarrhea alternating with constipation has also been reported; this is not uncommon in a number of protozoal gastrointestinal infections. The majority of infected individuals have intermittent diarrhea for 2 to 3 weeks, and many complained of intense fatigue, as well as anorexia and myalgia, during the illness. The clinical presentation of patients infected with this organism is similar to that of patients infected with *Cryptosporidium* spp.

It is highly unusual to see this organism in sputum, and reports of such findings are very rare. It is suspected that the patient may have come in contact with either several potential sources of infection or may have acquired a large infective dose of oocysts as a single exposure.

The transmission of *Cyclospora* is thought to be fecal-oral, although direct person-to-person transmission has not been well documented and may not be a factor since sporulation takes a number of days. Outbreaks linked to contaminated water and various types of fresh produce (raspberries, basil, baby lettuce leaves, and snow peas) have been reported.

Diagnosis

In clean wet mounts, the organisms are seen as nonrefractile spheres and are acid-fast variable with the modified

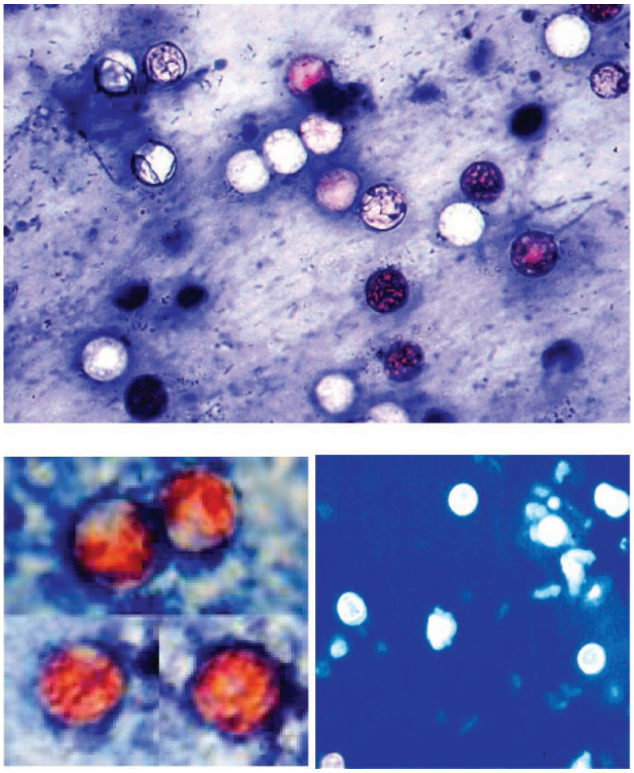


Figure 12.33 Case 22. (Upper) This image contains a modified acid-fast stain of stool using a 1 to 3% acid decolorizer (no stronger); *Cyclospora cayetanensis* oocysts, measuring approximately 8 to 10 μm, are seen. Notice that some stain and some remain clear (resemble wrinkled cellophane); thus they are modified acid-fast variable. Even with the 1% acid decolorizer, some oocysts may appear clear or very pale. Most laboratories are currently using 1% acid for the decolorizer. *Cyclospora* oocysts are not sporulated when passed, so no internal structures will be seen within the oocysts. (Lower, left) When a modified safranin technique is used, the oocysts uniformly stain a brilliant reddish orange if fecal smears are heated in a microwave during staining (courtesy CDC Public Health Image Library, photographed at a larger magnification [8 to 10 μm]); (right) note the autofluorescence of the oocysts; this preparation used the filters appropriate for calcofluor white. The autofluorescence can vary tremendously among clinical specimens; some oocysts may fluorescence at between ± to 3+. doi:10.1128/9781555819002.ch12.f33

acid-fast stain; those that are unstained appear as glassy, wrinkled spheres. Modified acid-fast stains stain the oocysts from light pink to deep red, some of which will contain granules or have a bubbly appearance (Fig. 12.33). It is very important to be aware of these organisms when the modified acid-fast stain is used for *Cryptosporidium* spp. and other similar but larger structures (approximately twice the size of *Cryptosporidium* oocysts) are seen in the stained smear. The acid decolorizer should be no stronger than 1 to 3% sulfuric acid; use of a stronger decolorizer may remove too much stain from the *Cyclospora* oocysts. It is important for laboratories to measure all acid-fast oocysts, particularly if they appear to be somewhat larger than those of *Cryptosporidium*. Under epifluorescence, the oocysts autofluoresce strong green (450 to 490 DM excitation filter) or intense blue (365 DM excitation filter). It is strongly recommended that during concentration (formalin/ethyl-acetate) of stool specimens, the centrifugation time and speed should be 10 minutes at 500 × *g*.

When a modified safranin technique is used, the oocysts uniformly stain a brilliant reddish orange if fecal smears are heated in a microwave during staining (Fig. 12.33, lower row, left). The stained slide can also be examined using epifluorescence microscopy first, and suspect oocysts can be confirmed using bright-field microscopy.

Key Points for Laboratory Identification

1. Specimens may appear as larger than normal *Cryptosporidium* oocysts; be sure to measure these organisms to confirm size.
2. Fecal specimens should be concentrated prior to staining; centrifugation for 10 minutes at 500 × *g* is recommended.
3. On wet smears, the oocysts will appear as nonrefractile spheres and will autofluoresce from bright green to intense blue with epifluorescence.
4. On modified acid-stained smears, the oocysts will stain from light pink to deep red, some of which will contain granules or have a bubbly appearance. Those that do not stain may have a wrinkled appearance.
5. *Cyclospora* is nonsporulated when passed; no internal structures will be visible within the oocysts.

Case 23

The microbiology section has just decided to incorporate immunoassays for *Giardia lamblia* and *Cryptosporidium parvum* into the laboratory test menu. The laboratory is also considering the addition of tests for the *Entamoeba histolytica/Entamoeba dispar* group and the true pathogen, *Entamoeba histolytica*. What factors should be considered prior to implementation of these immunoassays? Please comment on the issues presented below.

Test kit options and specimen collection requirements
Test ordering menu
Test coding
Test report formats
Client education
Personnel expertise
Geographic area

TABLE 12.2 Test kits for *Giardia*, *Cryptosporidium*, and *E. histolytica*/*E. dispar* group

Test kit options	Pros	Cons
Enzyme immunoassay (EIA)	Easy to use, can batch test; instrument read results recommended to eliminate problem of color interpretation	Minor color differences may be difficult to interpret; thorough washing mandatory
Fluorescent antibody (FA)	Easy to read; combination reagent available for both *Giardia* and *Cryptosporidium*; visual identification of cysts and oocysts (different sizes and shapes); can batch test; recommended to run on centrifuged material (enhances sensitivity); remember that pale *Giardia* trophozoites may occasionally be seen; this finding should be interpreted as a positive result.	Requires fluorescent microscope; cost may be a factor
Cartridge, EIA, or chromatographic IA (Rapid)	Easy to use and read; combination reagents available for *Giardia*, *Cryptosporidium*, *E. histolytica*/*E. dispar* group, or *E. histolytica*; can batch test; remember that testing for the *E. histolytica*/*E. dispar* group or *E. histolytica* must be performed using fresh or frozen fecal material (check package insert).	Can clog (fluid will not flow on the membrane) if particulate material is inoculated into the well

It is important to remember that the immunoassay tests currently available are not designed to “take the place” of the O&P examination (direct wet smear [fresh specimen only], concentration, and permanent stained smear). Both the O&P examination and the immunoassays for intestinal protozoa should be separate orderable, billable tests. Both options should be included in the test menu.

Discussion

Because there are so many variables to consider when introducing new tests into the laboratory, it is reasonable to assume that not every laboratory will offer the same test menu. There are very specific reasons for selection and/or nonselection of new methods. Some of these factors include geographic location and presence of the parasites being considered, patient populations (local, national, and/or international), size of the laboratory and number of specimens submitted, clients serviced, number of tests ordered, availability of trained personnel, trained consultants for troubleshooting technical problems, client educational options, etc. If we examine the various issues surrounding the list presented above, this information may help you in assessing whether or not your laboratory should introduce newer testing options for some of the intestinal protozoa. Although the kit names sometimes use *Cryptosporidium parvum*, it is well recognized that these tests recognize a number of different *Cryptosporidium* species. Thus, for accuracy, the results should report *Cryptosporidium* spp.

Test Kit Options and Specimen Collection Requirements. All immunoassay kits can be used with fresh or frozen specimens; however, only the kit formats that include *G. lamblia* and *C. parvum* can be performed using formalinized specimens (5% or 10% formalin, sodium acetate-formalin-acetic acid [SAF], and some of the single-vial collection systems) (TOTAL-FIX). In some instances, fecal specimens in Cary-Blair can be used; check each kit package insert for information. If testing is performed for the *E. histolytica*/*E. dispar* group or the true pathogen, *E. histolytica*, fresh or frozen specimens **only** can be used (Table 12.2).

Kit Sensitivity and Specificity. All available commercial immunoassay kits for the detection of antigen or actual organisms (cysts and/or oocysts) have published reports of both sensitivity and specificity of approximately 95% or better. A positive immunoassay result does **not** require confirmation testing by the O&P exam, and is **not** recommended for this purpose.

Test Ordering Menu. Depending on the patient history, it may be appropriate to order immunoassays for very specific organisms. However, it may be more important to order the O&P examination, particularly if the patient has been in a geographic area where parasites other than *Giardia* and/or *Cryptosporidium* may be present. Remember, these tests should always be separate orderable, billable tests. Refer to Table 12.3 for detailed information on specimen ordering recommendations.

Test Coding. It is important for billing purposes to use separate codes for the O&P examination, as well as separate codes for the enzyme immunoassay (EIA), fluorescent-antibody assay, or cartridge immunoassays. Specific coding information for diagnostic parasitology methods can be found in the Appendix section at the end of the book.

Test Report Formats. When reporting results of the O&P examination, it is important to inform the physician that *Cryptosporidium* spp. (with rare exceptions), *Cyclospora cayetanensis* and the microsporidia will not be detected from the routine O&P examination; special procedures must be ordered (modified acid-fast stains for the coccidia and modified trichrome stains for the microsporidia). When reporting results of the stool immunoassays, it is important to inform the physician that these procedures are designed to detect certain organisms **only**. Indicate that a negative report means that specific organisms (name the organisms) were not seen.

Client Education. Since the laboratory does not have sufficient patient information on which to base laboratory orders, it is mandatory that the physician clients have appropriate and complete information related to test ordering options. They should thoroughly understand the pros and cons of the O&P examination compared with the immunoassays; specific orders for one or the other are relevant, depending on the patient history. Some of the considerations include the patient's immune status, geographic area, travel history, and a history of day care. One of the most important considerations is the following: **if the patient becomes asymptomatic at the point at which negative laboratory results are reported, no further testing is required; if the patient remains symptomatic, then additional testing is warranted.**

Personnel Expertise. The performance of stool immunoassay testing is not difficult, providing the manufacturer's directions are followed. Thorough washing during EIA testing will eliminate the possibility of incorrect results; particulate matter should be avoided when performing antigen testing (EIA, Rapids). Known positive and negative specimens should be tested as a part of the learning phase.

Geographic Area. Recommendations are based partly on the location of the laboratory and travel history of the patients (Table 12.2). Again, it is important to provide specific guidelines on test ordering options for the physicians; adequate information will help ensure appropriate laboratory testing and clinically relevant information for quality patient care.

TABLE 12.3 Approaches to stool testing: recommended orders

Patient and/or situation	Test ordered[a]	Follow-up test ordered
Patient with diarrhea and AIDS or other cause of immune deficiency Potential waterborne outbreak (municipal/city water supply)	*Cryptosporidium* or *Giardia*/*Cryptosporidium* immunoassay	If immunoassays are negative and symptoms continue, special tests for microsporidia (modified trichrome stain) and other coccidia (modified acid-fast stain) and O&P exam should be performed.
Patient with diarrhea (nursery school, day care center, camper, backpacker) Patient with diarrhea and potential waterborne outbreak (resort setting) Patient with diarrhea from areas where *Giardia* is the most common parasite found	*Giardia* or *Giardia*/*Cryptosporidium* immunoassay (perform testing on two stools before reporting as negative—required for giardiasis, but not cryptosporidiosis). **Particularly relevant for areas of the U.S. where *Giardia* is the most common organism found**	If immunoassays are negative and symptoms continue, special tests for microsporidia and other coccidia (see above) and O&P exam should be performed.
Patient with diarrhea and relevant travel history Patient with diarrhea who is a past or present resident of a developing country Patient in an area of the United States where parasites **other than *Giardia*** are found	O&P exam, *Entamoeba histolytica*/*E. dispar* immunoassay; immunoassay for confirmation of *E. histolytica*; various tests for *Strongyloides* may be relevant (even in the absence of eosinophilia).	If exams are negative and symptoms continue, special tests for coccidia and microsporidia should be performed.
Patient with unexplained eosinophilia and possible diarrhea; if chronic, patient may also have history of respiratory problems (larval migration) and/or sepsis or meningitis (hyperinfection)	Although the O&P exam is a possibility, the agar plate culture for *Strongyloides stercoralis* (more sensitive than the O&P exam) is recommended; if available, molecular testing for strongyloidiasis is recommended; antibody serology for strongyloidiasis may also be helpful.	If tests are negative and symptoms continue, additional O&P exams and special tests for microsporidia and other coccidia should be performed.
Patient with diarrhea (suspected food-borne outbreak)	Test for *Cyclospora cayetanensis* (modified acid-fast stain, autofluorescence)	If tests are negative and symptoms continue, special procedures for microsporidia and other coccidia and O&P exam should be performed.

[a] Depending on the particular immunoassay kit used, various single or multiple organisms may be included. Selection of a particular kit depends on many variables: clinical relevance, cost, ease of performance, training, personnel availability, number of test orders, training of physician clients, sensitivity, specificity, equipment, time to result, etc. Very few laboratories will handle this type of testing exactly the same. Many options are clinically relevant and acceptable for good patient care. It is critical that the laboratory report indicate specifically which organisms could be identified using the kit; a negative report should list the organisms relevant to that particular kit.

Case 24

The microbiology laboratory has just received the quarter results and discussion for their proficiency testing in parasitology. Unfortunately, they missed the correct answer on one of the challenges. The particular specimen was a vial submitted for wet mount examination; no permanent staining was required. The laboratory had submitted a result of "No parasites seen"; unfortunately, the correct result was *Balantidium coli*.

Based on the results, can you think of any reasons why the correct answer for this specimen could have been missed? You may want to consider the following: specimen format, handling approach, wet mount preparation, microscopic examination, and reporting options.

In discussing the results among the staff, it appeared that the specimen had been examined correctly; however, this did not explain the discrepancy in results. After reviewing the information presented below, they had a better understanding of the issues and possible reasons for submitting the incorrect response.

Specimen Format. Proficiency testing specimens submitted as vials containing a 10% formalin/feces mix are designed to be examined as wet mounts; no permanent stains are required unless the participant is specifically instructed to prepare one of the special stains (modified acid-fast stain). However, for the most part, the liquid vials are to be examined as wet mounts only; fecal concentration is neither required nor recommended.

Handling Approach. Proficiency testing directions often indicate the vial should be shaken prior to pipetting some of the specimen for examination. However, even with few to moderate parasites present, there is always a chance that the fluid sampled might be reported as negative, particularly if the participant did not examine the wet mount correctly. Also, sampling error is always a possibility. Therefore, it is recommended that the vial be allowed to stand for 5 to 10 min after shaking so the organisms present can sink to the bottom of the vial. When sampling the liquid, place the pipette at the bottom of the vial prior to taking the aliquot. If you accidentally release bubbles from the pipette tip prior to sampling the specimen, allow the vial to stand another 5 to 10 min and try again. The proficiency testing specimens go through rigorous testing prior to being shipped to the participants. However, to enhance your chances of getting a good aliquot for the wet mount preparation, following these simple directions will help ensure that enough organisms will be present for an accurate identification.

Wet Mount Preparation. Based on numerous examinations prior to shipment, proficiency testing vials contain a reasonable number of the test parasites (with the exception of specimens intended to be negative). Therefore it is unlikely that the participant will prepare a wet mount that is too thin or too thick. However, the amount of fluid under the coverslip should cover the entire area (22 mm × 22 mm). It is also important not to add too much fluid, since the objects under the microscope will tend to shift position during the microscope examination or the excess fluid will flow onto the microscope stage.

Microscopic Examination. Scan the suspension (with or without iodine) with the 10× objective. The entire coverslip area should be examined (22 mm × 22 mm). Use the 40× objective for more-detailed study. At least one-third of the coverslip should be examined with the 40× objective, even if nothing suspicious has been seen at the lower magnification. Protozoan trophozoites and/or cysts and helminth eggs and larvae may be seen and identified. The microscope light should be reduced for low-power observations, since most organisms will be overlooked with bright light. Illumination should be regulated so that some of the cellular elements in the feces show refraction. Most protozoan cysts will refract under these light conditions. If too much iodine is used, large objects like helminth eggs and *B. coli* may be misidentified as artifact material.

Reporting Options. It is very important to familiarize yourself with the proficiency testing paperwork, particularly the reporting instructions. In the event the wrong organism number is entered or the information is entered in the wrong place, the participant result will probably be graded unacceptable. Therefore, it is critical to enter your results correctly to avoid penalties in the grading process.

Overall Discussion on Case History. If there was nothing to indicate a problem with the specimen itself in the proficiency testing quarter results writeup, we can assume the vial contained sufficient organisms for the identification. In this case, the following issues might have been responsible for the "incorrect response" by the participants.

1. If the vial was shaken and the aliquot immediately taken without letting the vial stand for 5 to 10 min, the number of organisms in the aliquot could have been minimal, and they were missed during the microscopic examination.
2. The wet mount preparation could have been too thin, and the organisms were missed during the microscopic examination.
3. If iodine was added to the wet mount, the amount could have been excessive, thus rendering the organisms too dark. They would resemble artifacts or helminth eggs and would have been misidentified as "negative" or the wrong organism.

4. The wet mount might not have been thoroughly examined as recommended above. Therefore, if the number of organisms was too small, any organisms present might have been missed.
5. If the correct results were not entered onto the reporting form in the proper place, then the result could have been graded unacceptable.

Overall, paying attention to these tips may prevent future errors of the same type. Certainly proficiency testing is not perfect; however, in the event the error is due to an actual factor related to the specimen preparation and/or shipment and the results reflect this type of problem, the participants will usually not be graded on the challenge. For the most part, the number of trial examination/reviews each specimen challenge undergoes prior to shipment ensures the quality of the challenge is excellent.

Case 25

A 43-year-old male was admitted to the hospital with complaints of sporadic diarrhea that had become worse over an extended period of weeks. This patient had previously been diagnosed as having AIDS and had been receiving HAART (highly active antiretroviral therapy) for several years. Unfortunately, resistance to protease inhibitors in this patient was followed by a decrease in CD4 count and an increase in viral load. He was treated with supportive care, and various specimens were submitted for diagnostic testing. Special stains were ordered for the microsporidia (modified trichrome stain); however, the results were negative.

Because a microsporidial infection was strongly suspected, additional specimens and modified trichrome stains were ordered. While reviewing the diagnostic methods used, one of the specific recommendations for specimen processing was not used. The potential problem involved centrifugation of the specimen prior to taking the sample for staining. Can you think of any potential problems associated with centrifugation that could influence the outcome? Also, you may want to consider the following: specimen format, specimen processing, smear preparation and staining, microscopic examination, and reporting options.

Specimen Format. The specimen could have been received as a fresh stool or preserved in one of several fecal fixatives. Specimen submission in the following formats would have been acceptable: fresh, fixed in formalin, mercury substitute fixatives (with or without polyvinyl alcohol/PVA), or the Universal Fixative (TOTAL-FIX).

Specimen Processing. During the past few years, it has become widely accepted that special stains for either the coccidia and/or microsporidia are more sensitive for organism detection if the smears are prepared from concentration sediment. In the UCLA Clinical Microbiology Laboratory, we have generated data (unpublished) to indicate that centrifugation at 500 × *g* for 10 min dramatically increases the number of microsporidial spores available for staining (from the concentrate sediment). This is the same centrifugation time/speed used for all stool specimens, regardless of the suspected organism.

Smear Preparation and Staining. If the patient has severe watery diarrhea, there is less artifact material in the stool to confuse with the microsporidial spores; however, if the stool is semiformed or formed, the amount of artifact material is much greater and the spores are much harder to detect and identify. Also, the number of spores varies according to the stool consistency (generally, the more liquid the stool, the more spores will be present). However, remember that there is also a dilution factor if the stool is very liquid.

Avoid the use of wet-gauze filtration (an old, standardized method of filtering stool prior to centrifugation) with too many layers of gauze that may trap organisms and not allow them to flow into the fluid to be concentrated. It is recommended that no more than two layers of gauze be used. Another option is to use the commercially available concentration systems in which metal or plastic screens are used for filtration.

Because the microsporidial spores are so small (1 to 2.5 μm), it is better to prepare a thinner smear rather than making it too thick. Better staining and easier microscopic examination can be achieved with thinner smears prepared from centrifuged stool sediment.

Microscopic Examination. When the smear is thoroughly fixed and the stain is performed correctly, the spores are ovoid and refractile, with the spore wall being varying shades of pink to red. Occasionally, the polar tube can be seen either as a stripe or as a diagonal line across the spore (Fig. 12.34). The majority of the bacteria and other debris tend to stain blue. However, some bacteria and debris still stain red. It is important to review the peripheral areas of the smear; also remember that spores can sometimes be found in clusters.

The microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be used for all measurements on the microscope. The calibration factors for all objectives should be posted on the microscope for easy access (multiplication factors can be pasted on the body of the microscope). Although recalibration every 12 months may not be necessary, this will vary from laboratory to laboratory, depending on equipment care and use.

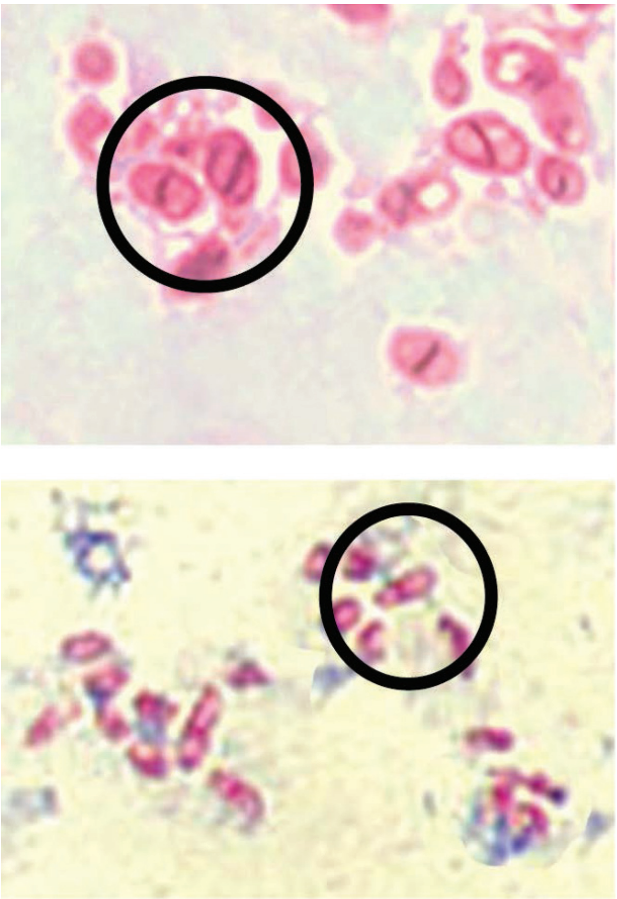


Figure 12.34 Case 25. Microsporidial spores stained with the modified trichrome stain (Ryan Blue). (Upper) Note the diagonal or horizontal lines within the spores, indicating the presence of the polar tubules (circle). (Lower) Spores photographed at a lower magnification; however, some of the polar tubules are visible (circle). Although these two images clearly demonstrate the spore and polar tubule morphology, some fecal smears are too thick and spores may not be visible.
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Reporting Options. The microsporidial spore wall should stain pinkish to red, with the interior of the spore being clear or perhaps showing a horizontal or diagonal stripe which represents the polar tube. The background appears green or blue, depending on the method. Other bacteria, some yeast cells, and some debris stain pink to red; the shapes and sizes of the various components may be helpful in differentiating the spores from other structures. The results of this staining procedure should be reported only if the positive control smears are acceptable. Immunoassay reagents should provide a more specific and sensitive approach to the identification of the microsporidia in fecal specimens (available outside of the United States; not FDA approved).

The organism can be reported as "Microsporidial spores present."

A report comment can be added as follows: The organisms are most probably *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis* (if from fecal specimen or urine).

Overall Discussion on Case History. The fact that the first specimen tested was reported as "No organisms seen" could be due to a number of factors indicated below.

1. If the smear was prepared from unspun stool, the number of spores may have been too low for organism detection. Preparation of the smear from concentrated/centrifuged sediment would have enhanced the overall sensitivity of the test.
2. If the smear was prepared too thick, organisms present could have been missed during the microscopic examination.
3. If the stained smear was not examined using the 100× oil immersion objective, the size of the organisms might have prevented detection without using the highest magnification available.
4. Even when the correct processing and examination methods are used, the microsporidial spores are shed sporadically. In the event of a negative result, it is recommended that another stool be submitted in a few days to a week later. Often, this second specimen is found to be positive.
5. If immunoassay reagents are available (currently no FDA-approved products are available within the United States); however, there are a number of fecal immunoassay kits available outside of the United States.

Overall, paying attention to the comments seen above may eliminate some of the potential errors in processing and examining specimens for microsporidia stained with the modified trichrome stains. However, due to the organism size, correct identification remains difficult, particularly when the specimen is stool that contains a lot of artifact material. Also remember, identification to the genus/species levels is not possible; the report would indicate "Microsporidial spores present."

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PART II

Clinically Important Human Parasites

13

Intestinal Nematodes

Ascaris lumbricoides

Enterobius vermicularis

Trichuris trichiura

Capillaria philippinensis

Hookworms (*Ancylostoma duodenale*, *Necator americanus*, and *Ancylostoma ceylanicum*)

Trichostrongylus spp.

Strongyloides spp.

The largest number of helminth organisms that parasitize humans is found in the roundworm group. Several roundworms are important in causing disease; some of these have other mammalian hosts, and some are considered to be pathogenic only under certain circumstances. Consequently, there is great variation in life cycle stages and pathologic sequelae found in humans.

Nematodes are unsegmented helminths with bilateral symmetry, have a fully functional digestive tract, are usually long and cylindrical, and vary from a few millimeters to over a meter long. Their numbers per patient vary considerably; however, worm size and numbers do not necessarily correlate with symptoms or pathologic changes.

All nematodes that are parasitic in humans have separate sexes, with the male usually being smaller than the female. Egg production varies considerably from species to species but tends to be consistent within a specific group. The number of eggs produced per day can range from a few (*Strongyloides stercoralis*) to more than 200,000 (*Ascaris lumbricoides*).

The life cycle of nematodes has five successive stages: four larval stages and the adult. In most cases, the third-stage larva is the infective stage. The eggs and larvae of nematodes living in the intestinal tract are passed outside the body in the feces or may be deposited on the perianal skin by the female worm. Eggs of certain species are almost fully embryonated, while other species may require an extended period of egg embryonation in the soil. In some cases, the eggs are infective when swallowed and other eggs will hatch in the soil, thus initiating infection by larval penetration of the human skin rather than egg ingestion.

As Table 13.1 shows, nematodes are long-lived worms. Usually, the host response to the presence of these worms is directly related to the worm burden. Diagnosis of these infections depends on the recovery and identification of adult worms, eggs, or larvae; specific information is provided in Table 13.2.

World Health Organization (WHO) estimates suggest that over 3.5 billion people carry nematode infections; filarial infections are not included in this figure. With these numbers in mind, it is not uncommon for almost any laboratory within the United States to occasionally recover helminth eggs, larvae, or adults from human specimens. Unfortunately, lowering the intensity of infection

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TABLE 13.1 Normal life spans of the most common intestinal nematodes

Nematode	Life span	Comments
Ascaris lumbricoides	1–2 yr	Infection may be aborted by spontaneous passage of adult worms.
Enterobius vermicularis	Several months to years	Reinfection due to both self-infection and outside sources is extremely common.
Trichuris trichiura	Several years	Often accompanies *Ascaris* infection (both are acquired by egg ingestion from contaminated soil)
Hookworms		
Necator americanus	4–20 yr	Symptoms are directly related to worm burden; many infections are asymptomatic.
Ancylostoma duodenale	5–7 yr	
Strongyloides stercoralis	30+ yr	Autoinfection capability can lead to dissemination and the hyperinfection syndrome in the compromised host.

in a host population through the use of chemotherapy may produce minimal declines in transmission relative to its initial endemic level.

It is well known that large numbers of people in sub-Saharan Africa are infected with both HIV-1 and intestinal helminths. There may be detrimental immunologic effects from coinfection with these two pathogens (1, 2). In patients with HIV/helminth coinfections, the issue of treatment remains somewhat controversial (1, 3).

Ascaris lumbricoides

The number of people infected with *Ascaris lumbricoides* worldwide is probably second only to the number infected with the pinworm, *Enterobius vermicularis. A. lumbricoides* was well known in Roman times as *Lumbricus teres* (confused with the common earthworm) and has probably been infecting humans for thousands of years. It is prevalent in moist, warm climates but can also survive in the temperate zones. In most countries of Central and South America, the average infection rates range up to approximately 45%. Also, foci of high prevalence among young children still persist in the southeastern United States (Table 13.3).

Life Cycle and Morphology

The adult worms are cylindrical, with a tapering anterior end. They are the largest of the common nematode parasites of humans; females measure 20 to 35 cm long, and males are 15 to 31 cm long, with a curved posterior end (Fig. 13.1). Also, the three well-developed lips are characteristic of this group.

The life cycle of *A. lumbricoides* is usually referred to as an “indirect cycle” as there is extensive migration throughout the body prior to adult worm maturation and egg production. Infection in humans is acquired through ingestion of the embryonated eggs from contaminated soil (Fig. 13.2). On ingestion, the eggs hatch in the stomach and duodenum, where the larvae actively penetrate the intestinal wall; they are then carried to the right heart via the hepatic portal circulation. Larvae within the eggs undergo one or possibly two molts prior to penetration of the intestinal wall. From the right heart they are carried into the pulmonary circulation, where they are filtered out by the capillaries. After approximately 10 days in the lung, the larvae break into the alveoli, migrate via the bronchi until they reach the trachea and pharynx, and then are swallowed. The worms then mature and mate in the intestine, with the eventual production of eggs, which are passed in the stool. The entire developmental process from egg ingestion to egg passage from the adult female takes from 8 to 12 weeks. During her life span, she may deposit a total of 60 million or more eggs.

Both unfertilized and fertilized eggs are passed (Fig. 13.3 and 13.4). Often only female worms are recovered from the intestine. Fertilized eggs become infective within 2 weeks if they are in moist, warm soil, where they may remain viable for months or even years. The fertilized egg is broadly oval, with a thick, mammillated coat, usually bile-stained a golden brown. These eggs measure up to 75 µm long and 50 µm wide. Unfertilized eggs are usually more oval, measure up to 90 µm long, and may have a pronounced mammillated coat or an extremely minimal mammillated layer. Often, both types of eggs are found in the same stool specimen. The total absence of fertilized eggs means that only female worms are present in the intestine.

The occurrence of *A. lumbricoides* usually peaks in childhood or early adolescence. Although the percentage of humans harboring *A. lumbricoides* is high, little is known about these infections in pregnant women and the possible impact on the fetus. In a clinical and epidemiologic study

TABLE 13.2 The most common intestinal nematodes

Characteristic	***Ascaris lumbricoides***	***Enterobius vermicularis***	***Trichuris trichiura***	**Hookworms (*Necator americanus*, *Ancylostoma duodenale*)**	***Trichostrongylus* spp.**	***Strongyloides stercoralis***
Usual time to infective stage	2–3 wk in soil; second-stage larva in egg	4–6 h; first-stage larva in egg	2–3 wk in soil; first-stage larva in egg	5–7 days in soil; free, third-stage larva	3–5 days in soil; free, third-stage larva	5–7 days in soil; free, third-stage larva
Mode of infection	Ingestion of infective egg	Ingestion of infective egg	Ingestion of infective egg	Skin penetration by *N. americanus*; ingestion and skin penetration by *A. duodenale*	Ingestion of third-stage larva	Skin penetration
Development and location in human host	Obligatory larval migration through liver and lungs; adults in small intestine	Direct development to adult in intestinal tract; adults in cecum, appendix, colon, and rectum	Direct development to adult in intestinal tract; adults in cecum, appendix, and colon	Larval migration through lungs; adults attached to mucosa of small intestine	Direct development to adult in intestinal tract; adults in small intestine	Larval migration through lungs; adult females in mucosal epithelium of small intestine; autoinfection may occur
Prepatent period	2 mo	3–4 wk	3 mo	6–8 wk	2–3 wk	2–4 wk
Normal life span	Up to 1 yr or slightly longer	1–2 mo	Up to 15 yr or more; usually 5–10 yr	Up to 15 yr or more; usually 5–10 yr	Up to 1 yr or slightly longer	Up to many years (30+)
Diagnosis by usual means	Bile-stained, mammillated, thick-shelled eggs (45–75 by 35–50 μm) in 1-cell stage in feces; infertile eggs (85–95 by 43–47 μm) have thinner shells and distorted mammillations; mature or immature adults may be found in feces or may spontaneously migrate out of the anus, mouth, or nares	Smooth, thick-shelled eggs (50–60 by 20–32 μm) in cellulose tape preparations; rarely seen in feces; adult or immature worms may be found in feces	Unembryonated, bile-stained, thick-shelled eggs (50–54 by 20–23 μm) with mucoid plugs at each end, in feces	Thin-shelled eggs (56–75 by 36–40 μm) in 4- to 16-cell stage in feces	Large, thin-shelled eggs (73–95 by 40–50 μm), tapered at one end, in feces; inner membrane of egg frequently wrinkled; ovum already in advanced cleavage when passed	First-stage larvae (108–380 by 14–20 μm) in feces; larvae have a short buccal cavity and a prominent, conspicuous genital primordium
Diagnostic problems	Fertile eggs may lose outer mammillated layer (decorticate eggs); infertile eggs may be difficult to recognize; also, will not float in usual solution of ZnSO4 (sp gr, 1.18) used for concentration	Eggs not usually seen in feces; cellulose tape method should be used to demonstrate eggs from perianal region	Rarely presents a problem; routine stool examination	Eggs of the 2 species are indistinguishable; if eggs hatch in feces owing to delay in examination, these first-stage larvae must be differentiated from *Strongyloides* larvae	May be confused with hookworm eggs	Larvae may be passed sporadically and may be found only by culture, concentration procedures, or use of Entero-Test or duodenal intubation
Clinical notes	Owing to potential migration of adult worms (as a result of fever, drugs, anesthetics), all infections should be treated; pulmonary symptoms may be present during larval migration (prior to egg recovery in the stool); eosinophils present but not impressive	Generally, only symptomatic patients treated because of high reinfection rate; there may or may not be eosinophilia	Light infections usually not treated; patients may be asymptomatic, with eggs an incidental finding; *Ascaris* and *Trichuris* infections often found together; moderate eosinophilia in heavy infections (usually will not exceed 15%)	Skin penetration by larvae produces allergic reaction (ground itch, cutaneous larva migrans); pulmonary symptoms usually present only in heavy infection; iron deficiency anemia and eosinophilia up to 70% may be present	Rarely seen in the United States; common in the Orient, Europe, Middle East, and Africa; light infections usually not treated	Hyperinfections may lead to death in the compromised or immunosuppressed host; patient may become symptomatic many years after original infection (without additional exposure); eosinophilia 10–40% or higher, but does not occur in every patient

of an infant with diarrhea and failure to thrive, the child became ill at 7 days of age, symptoms became worse at 21 days, and *Ascaris* eggs were found in the stool at 40 days. The minimum prepatent period after ingestion of the infective eggs is 60 days. After consideration of other possibilities, this case is very suggestive of congenital transmission. Further studies are necessary but, if confirmed, these findings will certainly reinforce the recommendations for antiparasitic treatment of pregnant women to prevent neonatal infection.

TABLE 13.3 Factors related to continuing *Ascaris lumbricoides* infections in urban communities in developing countries and DALYs lost to a range of causes, including ascariasis[a]

Factor	Value
Estimates for 1990[b]	
Population	2,500 million
Daily fecal output of population	500,000 tons
No. infected with *A. lumbricoides*	1,000 million
Daily fecal output contaminated with eggs of *A. lumbricoides*	200,000 tons
Daily discharge of eggs of *A. lumbricoides*	2×10^{14} eggs
Population of urban communities	750 million
Daily fecal output of urban population	150,000 tons
Number of urban people infected with *A. lumbricoides*	300 million
Daily fecal output contaminated with eggs of *A. lumbricoides* in urban communities	60,000 tons
Daily discharge of eggs of *A. lumbricoides*	6×10^{13} eggs
Estimates for urban communities in the year 2000[b,c]	
Population	2,200 million
Daily fecal output	440,000 tons
No. infected with *A. lumbricoides*	880 million[d]
Daily fecal output contaminated with eggs of *A. lumbricoides*	1,760,000 tons
Daily discharge of eggs of *A. lumbricoides*	1.76×10^{14} eggs
Comparison of DALYs[d] lost to a range of causes globally	
Total intestinal helminths	39 million
Ascaris lumbricoides	10.5 million
Trichuris trichiura	6.4 million
Hookworms	22.1 million
Malaria	35.7 million
Schistosomiasis	4.5 million
Tuberculosis	46.5 million
Measles	34.1 million
Vitamin A deficiency	11.8 million
Diabetes mellitus	8 million
Total maternal causes (hemorrhage, sepsis, eclampsia, hypertension, obstructed labor, and abortion)	29.7 million
Motor vehicle accidents	31.7 million

[a] Adapted from Coombs I, Crompton DWT, How much human helminthiasis is there in the world?, *J Parasitol* **85**:397–403, 1999; Bundy DAP, This wormy world—then and now, *Parasitol Today* **13**:407–408, 1997; and Chan MS, The global burden of intestinal nematode infections—fifty years on, *Parasitol Today* **13**:438–443, 1997.

[b] The world's population in 1990 was about 5,000 million, with about half living in developing countries (about 70% rural and 30% urban). About 1,000 million people in developing countries are infected with *A. lumbricoides*. About 200 g of stool is produced per person daily. About 1,000 eggs of *A. lumbricoides* are present daily in each gram of stool from an infected person.

[c] Probably underestimated and could be as high as 1,565 million.

[d] DALY, disability-adjusted life year; based on disabilities experienced translated into years of health lost.

Clinical Disease

Infection with *A. lumbricoides* continues to be a significant health problem throughout the world. Prevalence data in one of the first studies of its kind demonstrate that roundworm infection occurs at a high level in the population examined. There is a high within-individual correlation over time, indicating that there is individual predisposition to infection with *A. lumbricoides*. There is unequivocal evidence for a genetic component, accounting for 30 to 50% of the variation in worm burden. Shared environmental effects account for 3 to 13% of the total phenotypic variance. The remarkable consistency of the results through time and across different measures of the burden phenotype strongly indicates their validity and suggests the utility of future evaluations focused on identifying the specific genes responsible for these sizable genetic effects. Many of the parasitic helminth infections lend themselves to genetic analysis, and future studies may lead to significant improvements in pharmacologic agents (4).

Pathogenesis caused by *Ascaris* infections is attributed to (i) the host immune response, (ii) effects of larval migration, (iii) mechanical effects of the adult worms, and (iv) nutritional deficiencies due to the presence of the adult worms. Although the initial passage of larvae through the liver and lungs usually elicits no symptoms, there can be signs of pneumonitis if the number of larvae is quite large. When the larvae break out of the lung tissue and into the alveoli, there may be some damage to the bronchial epithelium. With reinfection and subsequent larval migrations, there may be intense tissue reactions, even with small numbers of larvae (Fig. 13.5). There may be pronounced tissue reaction around the larvae in the liver and lungs, with infiltration of eosinophils, macrophages, and epithelioid cells. This condition has been called *Ascaris* pneumonitis and is accompanied by an allergic reaction consisting of dyspnea, a dry or productive cough, wheezing or coarse rales, fever (39.9 to 40.0°C), transient eosinophilia, and a chest X ray suggestive of viral pneumonia. This picture of transient pulmonary infiltrates that clear within a couple of weeks and are associated with peripheral eosinophilia is frequently called Loeffler's syndrome. In addition to eosinophils and Charcot-Leyden crystals, the sputum may

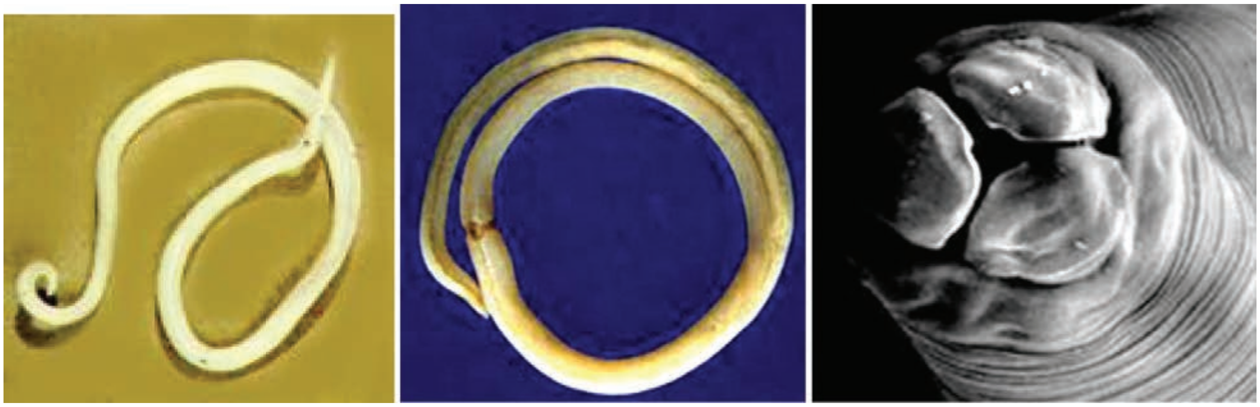


Figure 13.1 Adult *Ascaris lumbricoides*: (left) male; (middle) female; (right) three lips on anterior end. (Courtesy of CDC Public Health Image Library.) doi:10.1128/9781555819002.ch13.f1

contain larvae, although this finding is not common and examination of gastric washings may be more helpful. Asthma and urticaria may continue during the intestinal phase of ascariasis (5).

Eosinophilic gastroenteritis is an inflammatory disease characterized by eosinophilic infiltration of the gastrointestinal tract accompanied by varying abdominal symptoms and usually by peripheral eosinophilia. Although the precise etiology of this condition has not been determined, allergies to certain allergens such as foods, drugs, and parasites have been repeatedly proposed as causing the disease. In one rare case, a woman who had extensive eosinophilic infiltration in the descending and rectal colon, with a high titer to IgG antibody against *Ascaris suum*, was treated successfully with prednisolone (6).

A case of hepatic ascariasis occurring in a 3-year-old child with acute lymphoblastic leukemia suggests that *Ascaris* worms not only can enter the biliary tree and cause hepatic ascariasis but also potentially can carry pathogens on their surface and provide an alternate portal of entry for severe infections in neutropenic patients. At autopsy, two hepatic parenchymal abscesses were seen in the right lobe, each containing *Ascaris* worms, which were also found in the duodenum, common bile duct, and right intrahepatic duct. Cultures also grew *Klebsiella* spp., *Enterobacter cloacae,* and *Candida albicans* (85). Another case involved a 25-year-old woman with sickle cell trait and a normal delivery 3 months earlier who presented with acute obstructive cholangitis with septicemia and multiple hepatic abscesses. After removal of 60 adult *Ascaris* worms, surgical drainage of the hepatic abscesses, and wide-spectrum antibiotic therapy, the patient recovered. This case also points to the potential complications that can be seen with *Ascaris* infections, particularly when they occur in areas where this infection is not endemic. Endoscopy was successful in 19 of 25 patients resistant to medical therapy. Surgery remains important in the management of ascariasis complicated by biliary or pancreatic strictures and stones or by the presence of worms in the gallbladder (7).

The presence of adult worms in the intestine usually causes no difficulties unless the worm burden is very heavy;

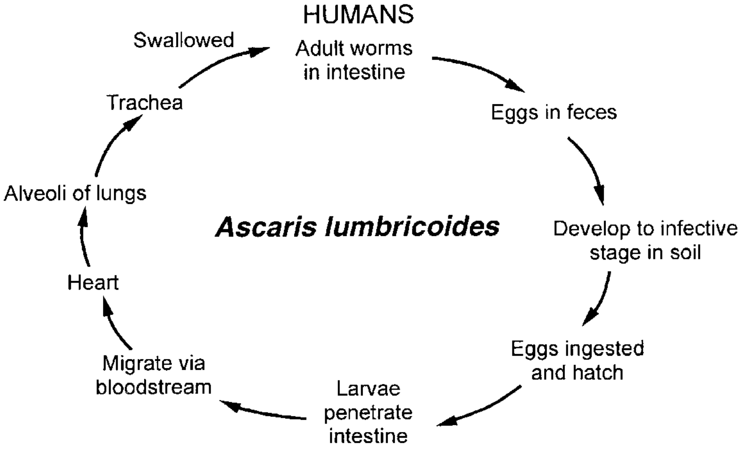


Figure 13.2 *Ascaris lumbricoides* life cycle. doi:10.1128/9781555819002.ch13.f2

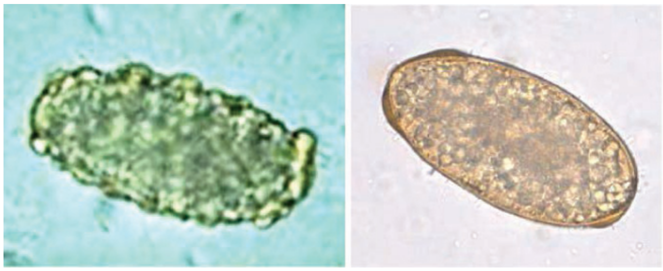
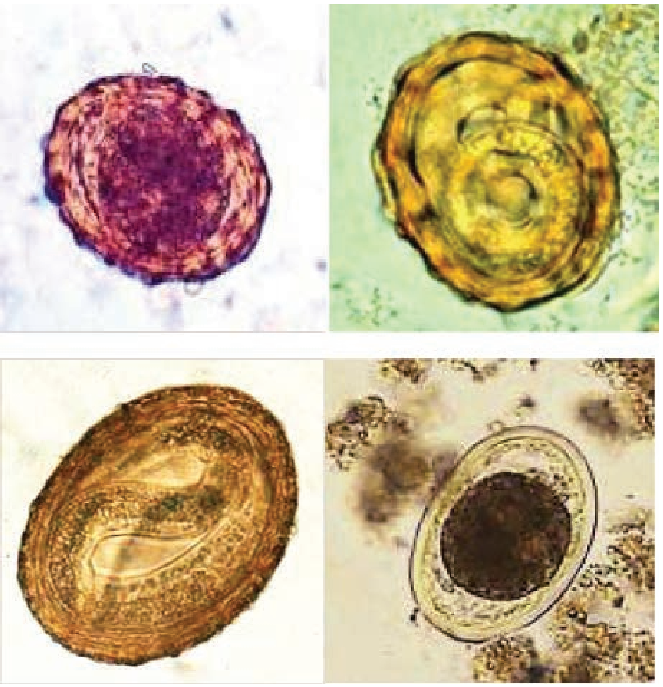


Figure 13.3 *Ascaris lumbricoides* unfertilized eggs. (Left) bumpy shell; (right) decorticate (loss of the bumpy coat); this can also be seen in the fertilized egg in Fig. 13.4.
doi:10.1128/9781555819002.ch13.f3

however, because of the tendency of the adult worms to migrate, even a single worm can cause serious sequelae (Fig. 13.6–13.9). Worm migration may occur as a result of stimuli such as fever (usually over 38.9°C), the use of general anesthesia, or other abnormal conditions. This migration may result in intestinal blockage; entry into the bile duct, pancreatic duct, or other small spaces; or entry into the liver or peritoneal cavity. The worms can also migrate out of the anus or come out the mouth or nose (Fig. 13.10). Other body sites, such as the kidneys, appendix, or pleural cavity, have been involved. A Swiss patient with acute pancreatitis and no evidence of gallstones or history of alcohol abuse died after a short fulminant illness. Autopsy findings revealed an *Ascaris* worm impacted within the ampulla of Vater, a reminder that nonindigenous causes of biliary tract obstruction need to be considered (8).

Figure 13.4 (Upper left) *Ascaris lumbricoides,* fertilized egg; (right) *A. lumbricoides,* fertilized egg containing larva. (Lower left) *A. lumbricoides* fertilized egg containing larva; (right) decorticate fertilized *A. lumbricoides* egg.
doi:10.1128/9781555819002.ch13.f4



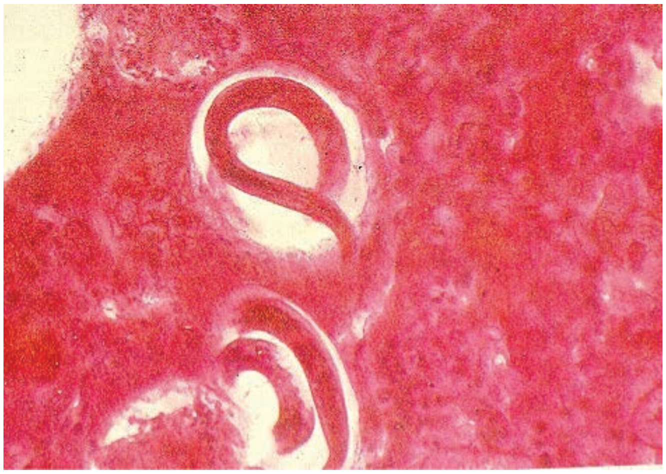
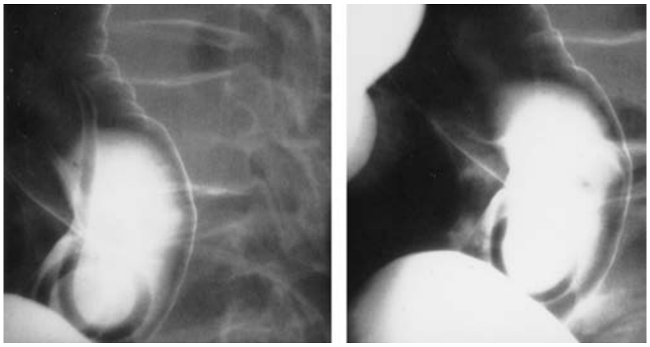


Figure 13.5 *Ascaris lumbricoides* larvae in lung tissue. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch13.f5

Through analysis of published reports, epidemiologic aspects of *Ascaris*-induced intestinal obstruction were identified. In nine studies of at least 100 patients admitted to the hospital with ascariasis, intestinal obstruction was the single most common complication and accounted for 38 to 88% of all complications. Both the proportion and number of cases per 1,000 population were significantly related to the local prevalence of ascariasis. In 12 studies of at least 30 patients with *Ascaris*-induced intestinal obstruction, the case fatality rates ranged from 0 to 8.6%.

Figure 13.6 *Ascaris lumbricoides*. Two spot films from an upper gastrointestinal barium study show that white barium in the small bowel outlines an adult worm that has displaced the barium and appears dark. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch13.f6



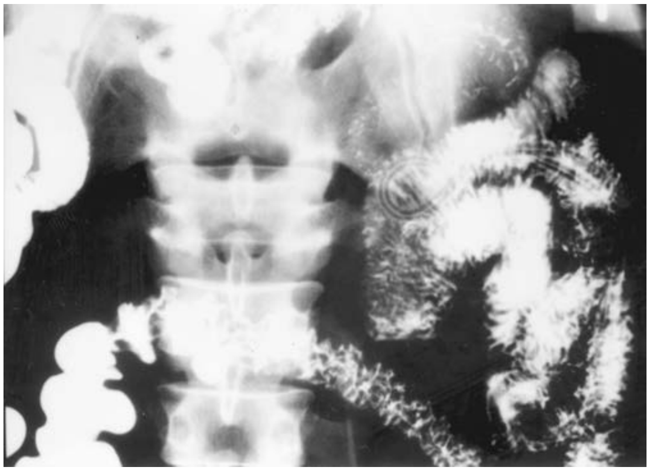
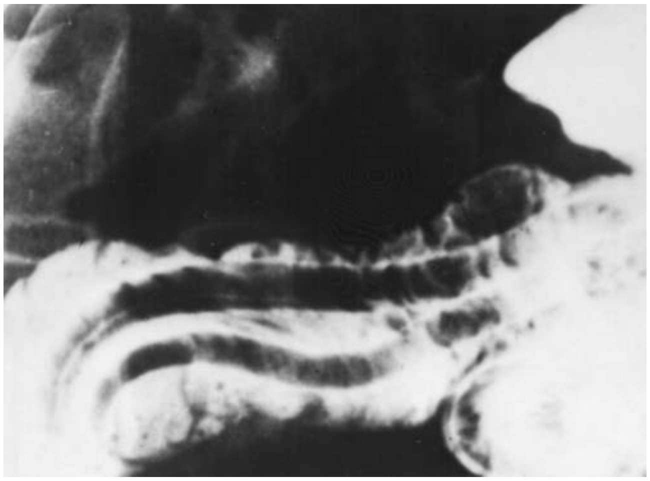


Figure 13.7 *Ascaris lumbricoides*. Small bowel barium study shows adult worms that present as longitudinal dark lines against the white barium in the small bowel. The worms have ingested the barium, and their guts appear as white lines between the dark body outlines. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch13.f7

The mean age of patients with obstruction was ≥5 years in six of seven studies in which age was specified (9).

In children, particularly those younger than 5 years, there may be severe nutritional impairment related to the worm burden. Directly measurable effects include increased fecal nitrogen and fecal fat and impaired carbohydrate absorption, all of which would return to normal with elimination of the adult worms. Worms can also be spontaneously passed without any therapy.

Figure 13.8 *Ascaris lumbricoides*, paired worms in the gut (X ray). (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch13.f8



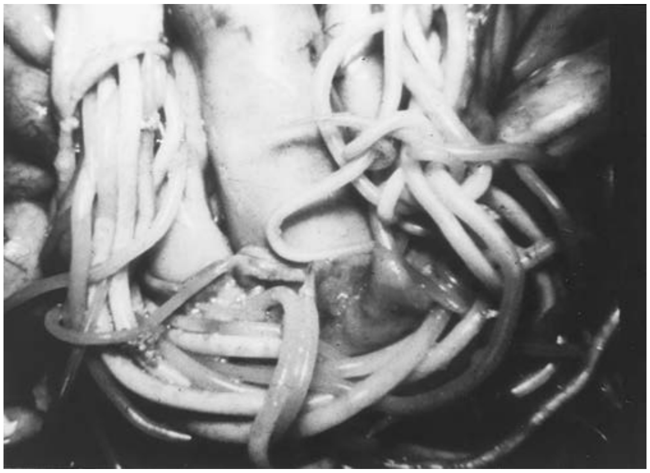


Figure 13.9 *Ascaris lumbricoides*, adult worms in the gut. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch13.f9

An association between helminthic infection and educational achievement has long been recognized. In one study, children treated with mebendazole for ascariasis showed significant improvement in several test scores, learning ability, concentration, and eye-hand coordination after 5 months of treatment (10). In another study, low test results in language, social, gross motor, and fine motor skills were associated with low weight for age, and lower language test scores were associated with infections with intestinal parasites and *Ascaris* in particular. Developmental disabilities are a significant and frequently undetected health problem in developing countries; malnutrition associated with intestinal helminth infections may be an important contributory factor to these disabilities (11).

Immunology and Host Resistance

Gastrointestinal nematode infections cause vigorous immune responses, although the mechanisms involved are not fully understood. A number of circulating immunoglobulins have been identified in *A. lumbricoides* infections, including IgG1 to IgG4, IgA, and IgE. The levels of IgG4 and IgE are elevated in helminth infections, and antiparasite IgE responses are associated with infection resistance. However, apparently IgG4 antibody can block IgE-mediated immunity and thus can block allergic responses in humans (12, 13).

The T-cell component of the immune system plays an important role in the resistance to gastrointestinal nematodes. Different nematode species may stimulate the same Th2 cascade, and functional resistance to certain species depends on different subsets of mediators and effectors within the cascade. Functional effectors at the base of the Th2 cascade probably include eosinophilia, IgA, IgE,

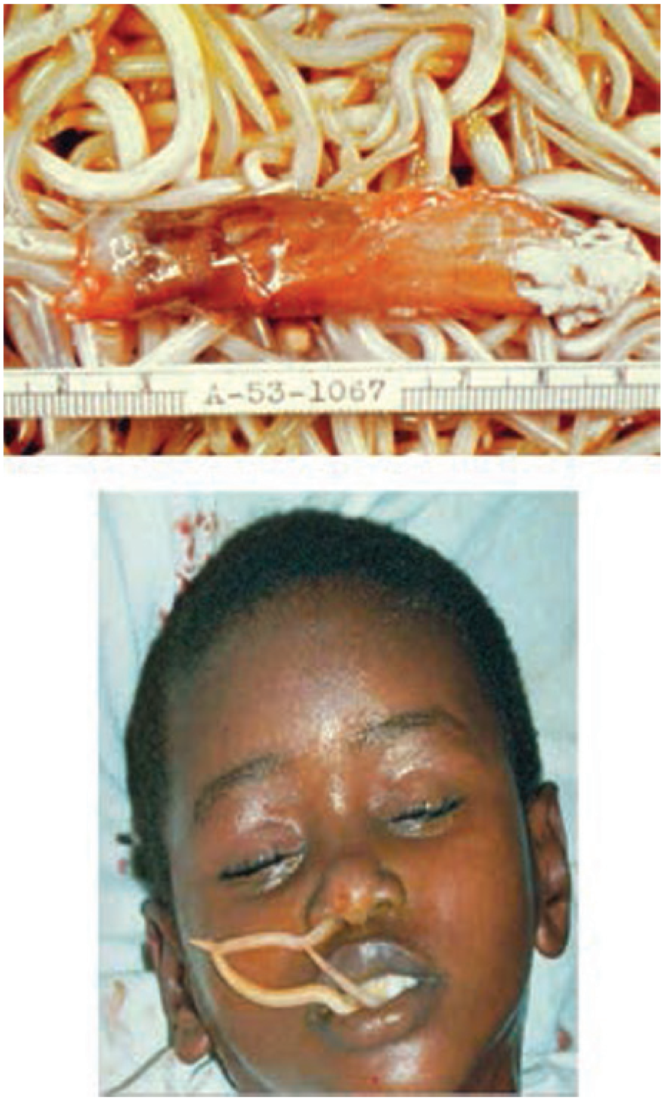


Figure 13.10 (Upper) *Ascaris lumbricoides* esophageal obstruction (courtesy of George Healy, CDC); (lower) adult *Ascaris* coming out of the nose and mouth. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch13.f10

proliferation of mast cells, and increased mucus secretion. Physiological changes in the intestine, such as increased mucosal permeability and smooth muscle contractility, may produce an environment in which worms become trapped in mucus and are swept from the gut; these changes are driven by interleukin-4 (IL-4) and IL-13.

It is well recognized that some helminth infections, including ascariasis, can suppress host immune responses. Worms may also subvert host immunity through the stimulation of inappropriate effectors. Various excretory-secretory products from parasitic nematodes can also modulate host immune responses. These products may interfere with immune effectors or influence mediators involved in induction and control. Overall, there remain many questions related to the deworming of human populations and the effect this could have on host susceptibility to certain pathogens (e.g., HIV) and on pathogen-induced immunopathology (12).

Diagnosis

In the larval migration phase of infection, diagnosis can be made by finding the larvae in sputum or in gastric washings. The typical Loeffler's syndrome is more likely to be seen in areas where transmission is highly seasonal.

During the intestinal phase, the diagnosis can be made by finding the eggs (unfertilized or fertilized) or adult worms in the stool (Fig. 13.3 and 13.4). The eggs are most easily seen on a direct wet smear or a wet preparation of the concentration sediment.

Caution. Remember that unfertilized *Ascaris* eggs do not float with use of the zinc sulfate flotation concentration method (the eggs are too heavy). Also, if too much iodine is added to the wet preparations, fertilized and unfertilized eggs may look like very dark debris. Eggs may be very difficult to identify on a permanent stained smear because of stain retention and asymmetric shape.

Intestinal disease can often be diagnosed from radiographic studies of the gastrointestinal tract, in which the worm intestinal tract may be visualized (Fig. 13.6 and 13.7). This may be particularly obvious when two worms are lying parallel, like "trolley car lines" (Fig. 13.8) (14).

Other involved body sites may present specific symptoms indicative of bowel obstruction, biliary or pancreatic duct blockage, appendicitis, or peritonitis (Fig. 13.9). Since acute abdomen caused by ascariasis is more commonly seen in developing countries, clinicians in developed countries may not consider this potential problem when seeing a patient with similar symptoms. Increased awareness of ascariasis presenting as an acute abdomen is required for optimal patient care; this may be possible only for an experienced radiologist (15). Therapeutic measures are related to specific symptoms and involved areas. Refer to Algorithm 13.1 for detailed information.

KEY POINTS—LABORATORY DIAGNOSIS

Ascaris lumbricoides

1. Both fertilized and unfertilized eggs can easily be recovered by the sedimentation concentration method. (Unfertilized eggs do not float with use of the zinc sulfate flotation concentration method.)
2. Because of the potential problems caused by migration of the adult worms, patients who are undergoing elective surgery and receiving general anesthetic should be checked for the presence of *A. lumbricoides* if there is any possible exposure history (some anesthetics stimulate the worms to migrate). Usually, a

(continued)

KEY POINTS—LABORATORY DIAGNOSIS

Ascaris lumbricoides (continued)

single stool examination will suffice to rule out the infection, unless only a single male worm is present (no eggs seen).

3. Larvae can be recovered from sputum (larval migration through the lungs); however, this is not a common finding.
4. Eggs may be very difficult to recognize on a permanent stained smear. They are usually very darkly stained and may be mistaken for debris. This problem also occurs with the addition of too much iodine when examining wet mounts; the eggs are obscured and resemble debris.

Treatment

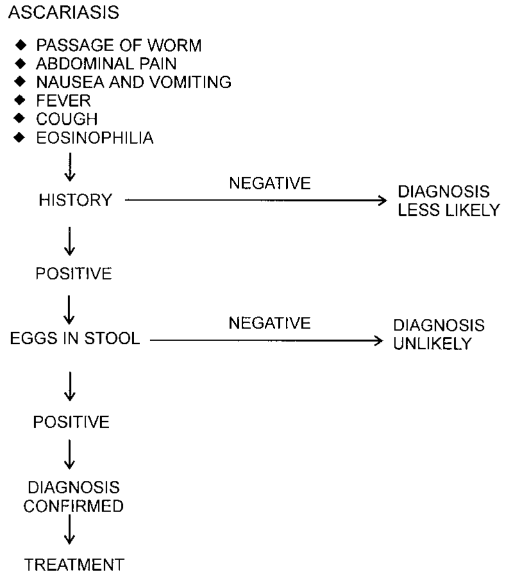
There are a number of modern anthelmintic agents that represent a vast improvement over some of the older remedies, and none of the recommended drugs require pre- or posttreatment purging or fasting. Albendazole or mebendazole is considered the drug of choice for both children and adults; ivermectin and pyrantel pamoate are alternatives but are considered investigational for this condition by the U.S. Food and Drug Administration. Specific drugs and dosages are listed in chapter 36 (16). Although these drugs are effective in eliminating the adult worms, there is no conclusive evidence that they are effective against the larval migration phase of the infection. If other body sites are involved, surgical intervention may be necessary.

The prognosis for treated intestinal disease is excellent; however, in cases of perforation or surgical procedures, there may be complications. Also, the prognosis may be extremely poor in cases of massive larval migration through the lungs.

Some individuals seem to be susceptible to heavy infections while others are not; deworming has a greater impact on the intensity of infection than on its prevalence; and mass chemotherapy is likely to be a more effective means of controlling morbidity than is selective treatment of heavily infected individuals only. Enhancing coverage and child-targeted treatment represent a more cost-effective approach than increasing the frequency of treatment. A study to compare the effects of mass, targeted, and selective chemotherapy with levamisole as an intervention for the control of ascariasis in areas of endemic infection revealed that, in terms of cost per 1,000-egg reduction in intensity and cost per person treated, the mass and targeted approaches were considerably more cost-effective than the selective approach (17).

Epidemiology and Prevention

Since the ultimate transmission of ascariasis depends on fecal contamination of the soil, the use of appropriate sanitary facilities is the primary means of prevention. There are apparently no practical means of killing the eggs while they are in the soil, especially when they are in clay soil under favorable environmental conditions (warmth and moisture). In some areas of the world where infections are common, some mass population treatment plans have been used with great success, even in areas with high reinfection rates. The use of human feces, or “night soil,” for fertilization of crops should be recognized as a potential hazard. Any vegetables or fruits from such fields cannot be eaten raw or unprocessed. Even with proper pretreatment of night soil, *Ascaris* eggs remain viable and infective more often than eggs of any other helminth species.



Algorithm 13.1 Ascariasis.
doi:10.1128/9781555819002.ch13.Alg13.1

In looking at the health of schoolchildren, four significant environmental influences on helminth infections have been identified: (i) an inadequate water supply, (ii) availability of school canteens, (iii) regular water/sanitation maintenance regimes, and (iv) overcrowded classrooms. Helminth infections are strongly associated with anemia, stunting and low weight, and the environmental conditions mentioned above. Although mass anthelmintic drug campaigns have taken place, reinfection is common and drug therapy alone is insufficient for elimination of the problem, particularly in a school environment (18).

Using *A. suum* as a model for *A. lumbricoides*, the ability of sludge treatment processes to kill the eggs of parasitic roundworms was examined. Both unembryonated and embryonated eggs were used, with interesting results. After 1 week in an anaerobic sludge digester, 95% of *A. suum* eggs produced two-cell larvae *in vitro*, with 86% progressing to motile larvae; after 5 weeks in the digester, 51% progressed to motile larvae. Between 42 and 49% of eggs stored in a sludge lagoon for 29 weeks were viable and able to develop motile larvae. Of embryonated eggs prior to treatment, >98% survived up to 5 weeks in an anaerobic sludge digester. More than 90% of embryonated eggs survived for 29 weeks in the sludge lagoon and were able to develop motile larvae (19).

Enterobius vermicularis

Enterobius vermicularis has been known since ancient times and has been studied extensively through the years; its original name was *Oxyuris vermicularis*. *E. vermicularis* is thought to cause the world's most common human parasitic infection. It has been said, "You had this infection as a child; you have it now; or you will get it again when you have children!" The infection is more prevalent in the cool and temperate zones, where people tend to bathe less often and change their underclothes less frequently. Prevalence in children can be high, a fact that has been recorded despite the difficulties in confirming the infection.

Although there has been some discussion of *Enterobius gregorii* being a distinct species of pinworm, it appears that this organism may actually be a young stage of *E. vermicularis*. A series of 849 male pinworms from a single individual were examined. Based on spicule morphology, the worms were classified into different groups. However, various transitional forms were observed in the spicule morphology in the worms with intermediate body size between *E. vermicularis* and *E. gregorii*, showing that the basal portion of the spicule of *E. vermicularis* develops after completion of the *E. gregorii*-type basal portion (20).

Life Cycle and Morphology

The female worm measures 8 to 13 mm long by 0.3 to 0.5 mm wide and has a pointed tail (hence the name "pinworm") (Fig. 13.11). The male is much smaller, measuring 2 to 5 mm long by 0.1 to 0.2 mm wide, and has a curved caudal end. Infection in humans is initiated by the ingestion of infective eggs, which hatch in the intestine (cecal region), where they develop into the adult worms (Fig. 13.12). It probably takes about 1 month for the female to mature and begin egg production. After fertilization of the female worms, the males usually die and may be passed out in the stool. In gravid females, almost the entire body is filled with eggs. At this point, the female migrates down the colon and out of the anus, where the eggs are deposited on the perianal and perineal skin. Occasionally, the female worm migrates into the vagina. It is speculated that, after egg deposition, the female worm returns to the intestine; however, this has not been proven (14). Occasionally, when the bolus of stool passes out of the anus, adult worms become attached to the stool and can be found on the surface. Adult worms can also sometimes be picked up on the Scotch tape preparations used to diagnose this infection. Although egg deposition usually does not occur in the intestine, some eggs may be recovered in the stool. The eggs are fully embryonated and infective within a few hours. Although transmission is often attributed to the ingestion of infective eggs by nail biting and inadequate hand washing, airborne eggs can also be inhaled and ingested.

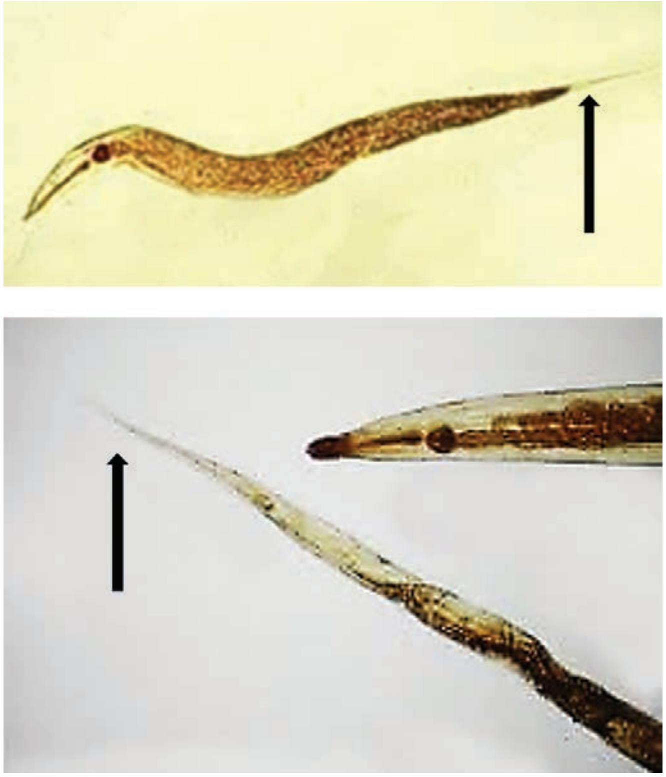
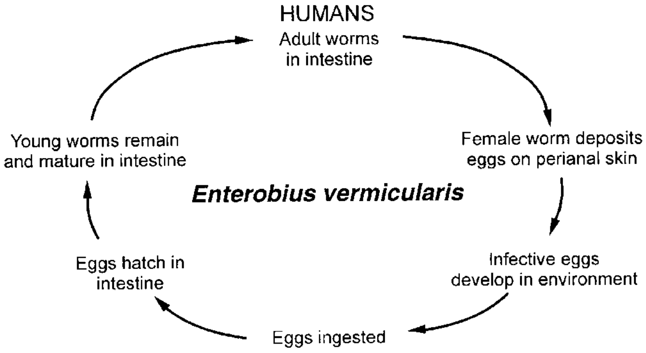


Figure 13.11 *Enterobius vermicularis*, adult female pinworms. Note pointed tail (arrows).
doi:10.1128/9781555819002.ch13.f11

The eggs tend to be oval, compressed laterally, and flattened on one side, and measure 50 to 60 µm long by

Figure 13.12 *Enterobius vermicularis* life cycle.
doi:10.1128/9781555819002.ch13.f12


HUMANS
Adult worms in intestine
Female worm deposits eggs on perianal skin
Infective eggs develop in environment
Eggs ingested
Eggs hatch in intestine
Young worms remain and mature in intestine
Enterobius vermicularis

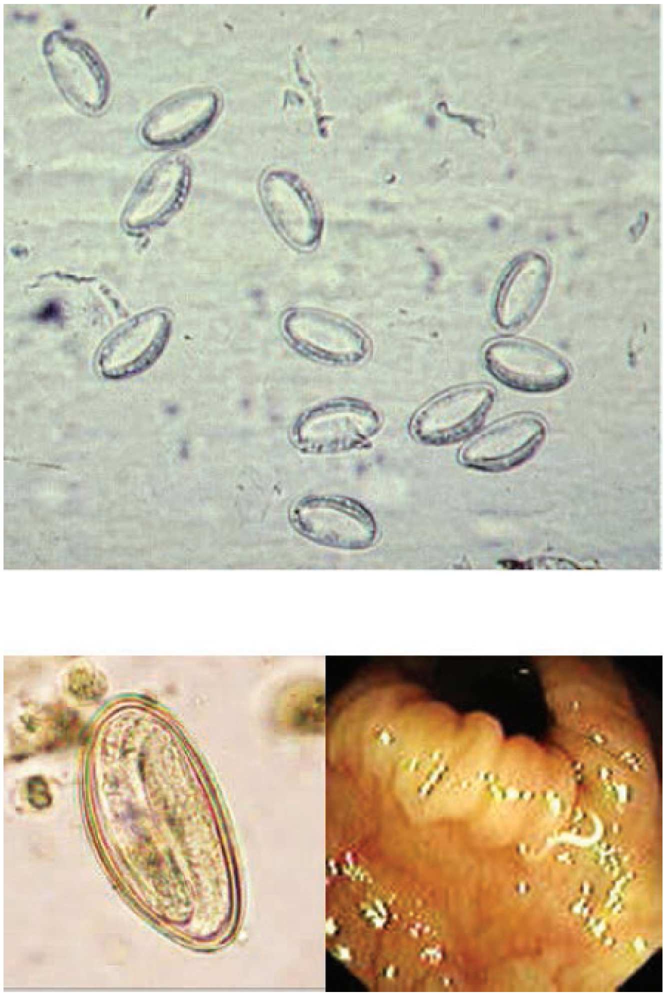


Figure 13.13 *Enterobius vermicularis*. (Upper) Eggs on a Scotch tape preparation. (Lower left) Enlarged egg containing larva; (right) adult worm seen on perianal skin.
doi:10.1128/9781555819002.ch13.f13

20 to 30 µm wide (Fig. 13.13). The eggs are somewhat sticky and can survive on surfaces for some time.

Clinical Disease

Pinworm infection is the most common helminth infection within the United States and Western Europe; prevalence rates in some areas may be as high as 30 to 50%. The most striking symptom of this infection is pruritus, which is caused by the migration of the female worms from the anus onto the perianal skin before egg deposition. The sometimes intense itching results in scratching and occasional scarification. In most infected people, this may be the only symptom, and many individuals remain asymptomatic.

The degree of infection varies tremendously from patient to patient. As many as 5,000 worms have been removed from a single patient; however, most cases average less than 1 migrating worm per evening. Women are symptomatic three times as often as men, and young people are symptomatic more frequently than older people.

Eosinophilia may or may not be present. Although tissue invasion has been attributed to the pinworm, these cases are not numerous. Infections tend to be more common in children and occur more often in females than in males. In heavily infected females, there may be a mucoid vaginal discharge, with subsequent migration of the worms into the vagina, uterus, or fallopian tubes, where they become encapsulated. Other symptoms have also been attributed to pinworm infection, particularly in children; they include nervousness, insomnia, nightmares, and even convulsions (14).

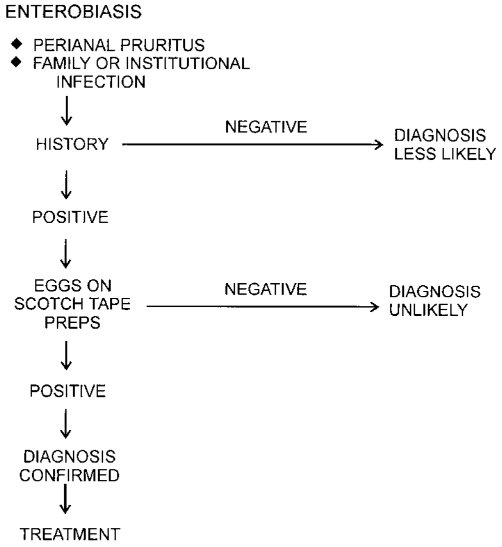
In one report, a homosexual man presented with severe abdominal pain and hemorrhagic colitis, eosinophilic inflammation of the ileum and colon, and numerous unidentifiable larval nematodes in the stool (21). By using morphologic characteristics and molecular cloning of nematode rRNA genes, the parasites were identified as larvae of *E. vermicularis*; these larvae are rarely seen and are not thought to cause disease. The authors state that occult enterobiasis is widely prevalent and may be a cause of unexplained eosinophilic enterocolitis (21). Although enterobiasis is an uncommon cause of acute appendicitis in children within the United States, this infection may be associated with acute appendicitis, chronic appendicitis, or ruptured appendicitis or with no significant symptoms (22).

Other uncommon ectopic sites have included the peritoneal cavity, lungs, liver, urinary tract, and natal cleft. Pathologic examination usually reveals chronic granulomatous inflammation with or without central necrosis, which is surrounded by polymorphonuclear neutrophilic leukocytes, eosinophils, and fibroblasts. Macrophages, giant cells, epithelioid cells, and Charcot-Leyden crystals may also be present. During a case of suspected transverse colon carcinoma, histologic examination of the mass revealed eggs of *E. vermicularis* embedded in granulomatous tissue in the submucosa of the colon; no malignancy was found (23). Apparently, this is the first report of enterobiasis presenting as colon carcinoma.

Diagnosis

Although the patient history of anal itching, irritability, and insomnia may suggest a pinworm infection, diagnosis depends on demonstrating the presence of eggs or adult worms. This is normally accomplished by sampling the perianal and perineal skin with cellulose tape (Scotch tape), which is applied sticky side down to the skin. The tape is transferred to a glass slide and examined under the microscope for the presence of eggs or adult worms (see chapter 5). Commercial paddles are also available for the collection of eggs and/or adult pinworms. Eggs are rarely found in the stool (approximately 5% of the time), and sampling of the perianal folds yields more accurate results. Since the female worms migrate on a sporadic basis, a series of four to six consecutive tapes may be necessary to demonstrate the infection. The tapes are used late in the evening, when the patient has been sleeping for several hours, or first thing in the morning before the patient takes a shower or goes to the bathroom. These samples can be taken from children at home and transported to the laboratory for examination. Refer to Algorithm 13.2.

In cases of ectopic infection, diagnosis usually requires biopsy and histologic examination. Although rare, histologic examination of tissue is particularly important in differentiating malignancy from parasitic infection. The typical appearance of the worm in cross section within the appendix can be seen in Fig. 13.14. Note the lateral alae at each side (indicated by the arrows).



Algorithm 13.2 Enterobiasis.
doi:10.1128/9781555819002.ch13.Alg13.2

KEY POINTS—LABORATORY DIAGNOSIS

Enterobius vermicularis

1. The cellulose tape (Scotch tape) preparation is recommended as the diagnostic test of choice (a minimum of four to six consecutive negative tapes is required to rule out the infection). Commercial paddles or other collection devices are also acceptable. Although the paraffin swab is also an option, this method is seldom used.
2. Adult worms may be found on or under the surface of the stool specimen, particularly in children. The adult worms can also be found on the tapes.
3. Eggs are occasionally recovered in stool, but this is an incidental finding and not the specimen of choice.

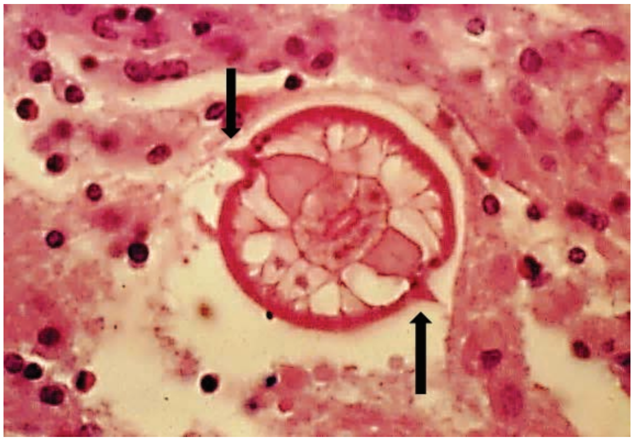
Treatment

Although several drugs such as albendazole or mebendazole are extremely effective in eliminating the worms, the decision to treat should be based on evidence of infection and consideration of whether the patient is symptomatic (16). Alternatives include ivermectin or pyrantel pamoate. Repeat reinfection is always possible; thus, repeated treatment may be necessary to ensure that the patient is free of infection. Repeat treatment after about 2 weeks is routinely recommended to eliminate any infection acquired from ingestion of eggs that remain in the environment following the first treatment. Asymptomatic individuals are rarely treated, particularly in a group situation in which more susceptible individuals are not present. Treatment often includes counseling for the parents, who may be very upset at learning that their children have “worms.” They may not realize how prevalent the infection is, particularly in children, and that many children never have any symptoms or sequelae of the infection. In a group situation, often only one or several individuals will be symptomatic and continue to be infected. Treatment of the entire group, followed by retreatment of the more susceptible individuals, may provide an infection-free group for a longer period.

Epidemiology and Prevention

Since this infection is so common and transmission is so easy (anus-to-mouth contamination, soiled night clothes, airborne eggs, and contaminated furniture, toys, and other objects), prevention is marginal. Improved personal and

Figure 13.14 *Enterobius vermicularis* cross section of adult worm in appendix. Note the pointed lateral alae (arrows).
doi:10.1128/9781555819002.ch13.f14



group hygiene combined with group therapy can be helpful. It is also recommended that children sleep in closed garments and maintain short, clean fingernails. Sunlight and UV lamp radiation will destroy the eggs in the environment, and dry heat can be used to sterilize metal toys. The eggs are not killed by the level of chlorination used in swimming pools. Recent studies also reveal that *Enterobius* infestation can occur in cockroaches, which may serve as potential reservoirs for these helminths and have considerable public health significance (12). *Under most circumstances, total prevention is neither realistic nor possible.*

Trichuris trichiura

Trichuris trichiura was first described by Linnaeus in 1771 and was studied extensively in the late 1880s and early 1900s. Two early names include *Trichocephalus trichiurus* and *Trichocephalus dispar*. Well-preserved *Trichuris* eggs were found in feces in the rectum of a 9-year-old Inca girl whose frozen body was recovered in a stone building at the 17,568-ft altitude near Santiago, Chile; it was calculated that the child had died approximately 450 years earlier (14). In 1991, a well-preserved mummy of a 25- to 30-year-old man was found in a glacier in the Alps at an altitude of 3,200 m; it was estimated that the man had died about 5,200 to 5,300 years earlier. In studies of material from the colon, preservation and processing of material in sodium acetate-acetic acid-formalin fixative revealed the presence of *T. trichiura* eggs, the oldest finding of *Trichuris* in a human (3200 to 3300 BCE) (24).

Infection with *T. trichiura* is more common in warm, moist areas of the world and is often seen in conjunction with *Ascaris* infections. Worm burdens vary considerably, and individuals with few worms are unaffected by the presence of these parasites. Prevalence rates of 20 to 25% have been reported from the southern United States (14).

Life Cycle and Morphology

Whipworms are much larger than pinworms, measuring 35 to 50 mm long (female) or 30 to 45 mm long (male); the male has a 360° coil at the caudal extremity (Fig. 13.15). The adult worms are rarely recovered from the stool, since they are attached to the wall of the intestine. The head portion of the worm is very thin and is embedded in the mucosa, while the posterior end is much thicker and lies free in the lumen of the large intestine. The large, posterior end has been described as the whip handle while the thin, anterior end is the whip itself, hence the name whipworm.

Human infection is acquired through ingestion of the fully embryonated eggs from the soil (Fig. 13.16 and 13.17). The eggs hatch in the small intestine and eventually attach to the mucosa in the large intestine. The adults mature in about 3 months and begin egg production. The eggs are barrel shaped with clear, mucoid-appearing polar plugs (Fig. 13.18). They measure 50 to 54 μm long and 22 to 23 μm wide. They are passed in the unsegmented stage and require 10 to 14 days in moist soil for embryonation to occur. Distorted eggs that are much larger than normal have been seen following therapy with mebendazole and with other drugs. This is not a common finding but is something to consider if distorted eggs are seen. There are also some reports in the literature that *T. vulpis* (dog whipworm) eggs have been recovered in human stools (25). These eggs tend to be larger (70 to 80 μm long by 30 to 42 μm wide) and have prominent but small polar plugs compared with those of *T. trichiura*. Several studies have also confirmed that larger eggs among others of normal size have been recovered in human stool in cases where the larger egg size was not associated with treatment. Studies also confirm that these larger eggs can develop to the infective stage (14).

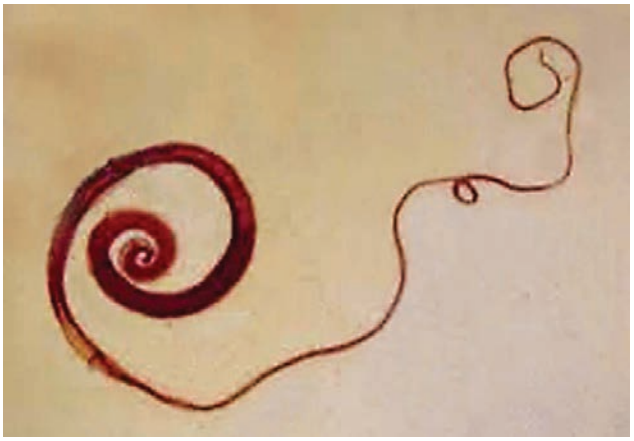


Figure 13.15 *Trichuris trichiura* adult worm. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.) Note: the small end is the head end; thicker portion of worm is the "handle."
doi:10.1128/9781555819002.ch13.f15

Clinical Disease

The differential diagnosis of chronic diarrhea requires consideration of several diseases, including celiac disease, inflammatory bowel disease, and irritable bowel syndrome. Patients infected with *T. trichiura* may present with a chronic dysentery-like syndrome if they have a massive infestation leading to anemia and growth retardation. In some cases, diarrhea may last for years without blood and mucus. However, once blood is evident, medical intervention may occur; in some cases the diagnosis requires colonoscopy. Also, in these cases, prolonged therapy may be necessary to eliminate the parasites (26).

Mechanical damage to the mucosa and the allergic response by the host appear to be the main reasons for

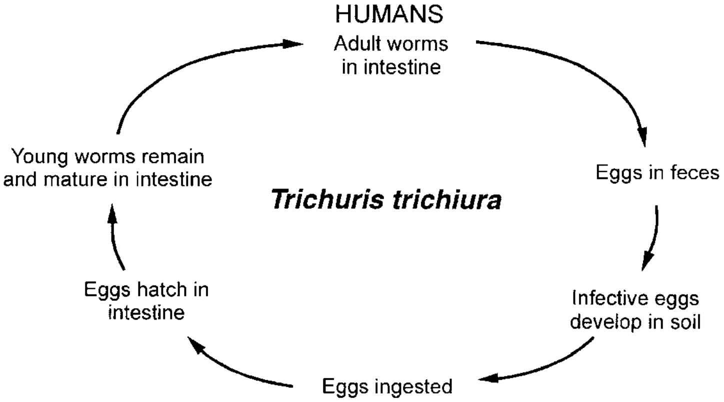


Figure 13.16 *Trichuris trichiura* life cycle. doi:10.1128/9781555819002.ch13.f16

any abnormalities associated with *T. trichiura* infection and are definitely related to the worm burden, the length of the infection, and the age and overall health status of the host. Although the worm is actually threaded into the epithelium of the cecum, damage produced by this process is minimal unless there is dysentery, during which the mucosa may be edematous and friable, and there may be abdominal cramps, severe rectal tenesmus, and rectal prolapse (Fig. 13.19 and 13.20). The dysentery seen in severe infections may mimic that seen in amebiasis. Most infections are light to moderate, with minimal or no symptoms.

Hypochromic anemia may be seen in patients with prolonged, massive infection; however, the anemia is due to malnutrition and blood loss from the friable colon and is not related to blood ingestion by the parasite. In a study of 409 Jamaican schoolchildren, iron deficiency anemia was associated with *Trichuris* infections involving over 10,000 eggs/g but not with less severe infections (27). Although eosinophils and Charcot-Leyden crystals are present in the stool in patients with dysentery, a peripheral eosinophilia on the differential smear is not always seen and the degree of eosinophilia may not correlate with the severity of infection (it rarely exceeds 15%). Heavy infections tend to be rare in developed countries, and complications requiring surgical intervention are also rarely described. A case of colonic obstruction and perforation has been described in an 84-year-old woman. She was originally admitted for a chest infection; 2 days after admission, she suffered from nausea and vomiting, followed by bowel stoppage 2 days later. A partial right-sided ileocolectomy was performed; pathology revealed a heavy infestation with *T. trichiura*, causing a pseudotumor following a proliferative inflammatory response (28).

There are few data on mucosal immune responses to intestinal helminths in humans, particularly involving the IgE system, which is thought to be important in parasite expulsion. Cooper et al. (29) did find evidence of an IgE-

Figure 13.17 *Trichuris trichiura* eggs. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch13.f17

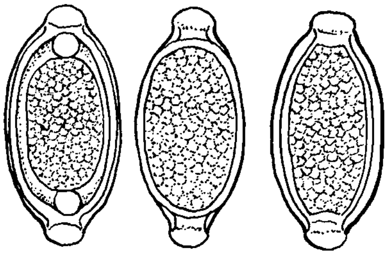
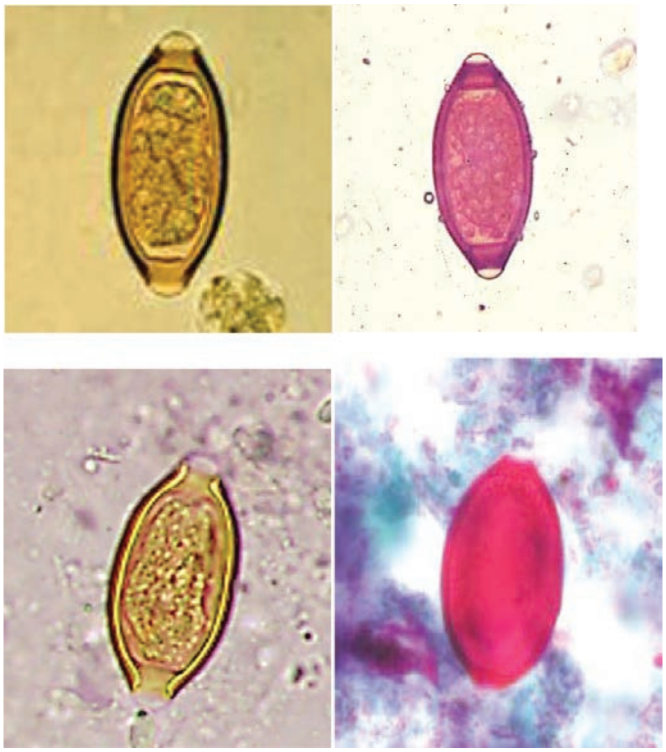


Figure 13.18 *Trichuris trichiura* eggs; note the two polar plugs, one at each end of the egg. (Lower right) Egg stained with Wheatley's trichrome (routine ova and parasite stain). doi:10.1128/9781555819002.ch13.f18



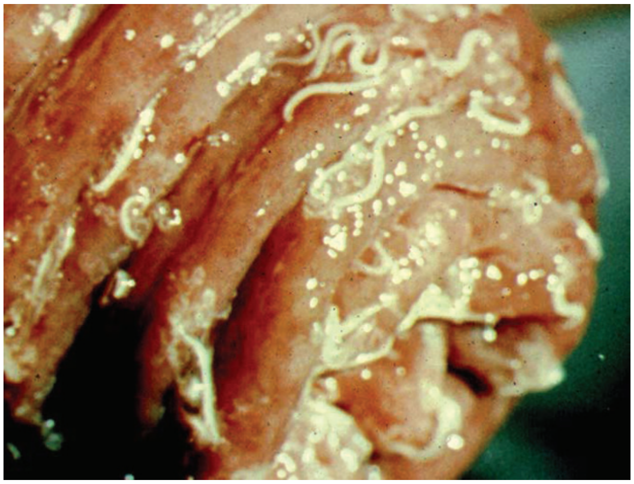
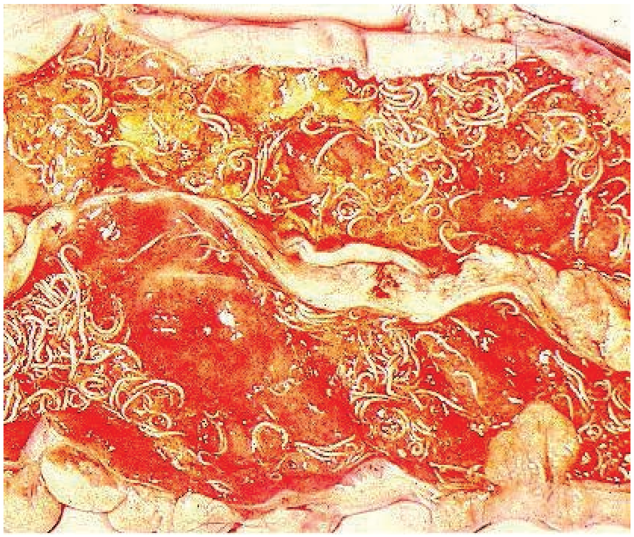


Figure 13.19 *Trichuris trichiura*, rectal prolapse. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch13.f19

mediated immune mucosal response to *Trichuris* infections, but it was not sufficient to cause parasite expulsion. On examination of rectal biopsy specimens, children with *Trichuris* dysentery syndrome had significantly greater numbers of mast cells and cells with surface IgE than did the control group. Unanswered questions involve possible antibody-mediated immunity and protection against invading larvae and whether *Trichuris* dysentery syndrome develops in some children because they have a defect in antiparasite cell-mediated immunity. It is probable that both cellular and humoral responses are required to eliminate the worms from the colon.

Figure 13.20 Numerous adult *Trichuris trichiura* worms attached to the colonic mucosa of a patient who died of trichuriasis. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch13.f20



A study in Jamaica was designed to determine whether *T. trichiura* dysentery is associated with evidence of a systemic inflammatory response and whether the plasma protein disturbance has special characteristics compared with the situation in uninfected children in the area of endemic infection (30). C-reactive protein, α-antitrypsin, total globulin, and fibronectin levels and plasma viscosity were significantly higher in children with *T. trichiura* dysentery. There appeared to be an acute-phase response in intense trichuriasis and a specific elevation of the fibronectin level in plasma. Also, plasma viscosity remained abnormally high 6 months after treatment, although it was lower than at diagnosis.

It appears that an increase in the rate of epithelial cell turnover in the large intestine may lead to the expulsion of *Trichuris*. This increased rate of epithelial cell movement is under immune control by the cytokine IL-13 and the chemokine CXCL 10 (31). Such a host protective mechanism may help explain the roles played by the intestinal epithelium in mucosal defense.

Trichuris suis Therapy for Active Ulcerative Colitis

Inflammatory bowel disease, including Crohn's disease, probably occurs from a failure to downregulate a chronic Th1 intestinal inflammatory process. Induction of a Th2 immune response by intestinal helminths reduces the Th1 inflammatory process. Ulcerative colitis is more common in Western industrialized countries than in underdeveloped countries, particularly those where helminth infections are common. People with helminth infections exhibit altered immunologic antigen responses. It has also been shown in animal models that helminths prevent or improve colitis through the induction of regulatory T cells and modulatory cytokines. The use of *T. suis* in the therapy of ulcerative colitis has been a controversial and debated issue for several years. A recent randomized, double-blind, placebo-controlled trial conducted at the University of Iowa and selected private practices has shown improvement in 43.3% of patients treated with ova compared with 16.7% who received placebo.

Although the benefit was temporary in some patients who received a single dose of 2,500 *T. suis* eggs, improvement could be sustained with maintenance therapy every 3 weeks. Consequently, this approach appears to be safe and effective in patients with active colitis (32). In another discussion, trials using helminths such as hookworm (*Necator americanus*) or porcine whipworm (*T. suis*) show that they are safe and may be effective therapies for the control of the aberrant intestinal inflammation seen in Crohn's disease and ulcerative colitis (33).

Diagnosis

Most whipworm infections can be easily diagnosed by finding the characteristic eggs in the stool. These eggs should be quantitated (rare, few, moderate, many), since light infections usually cause no problems and do not require therapy.

Note *T. trichiura* eggs submitted in stool preserved with polyvinyl alcohol do not concentrate as well as those preserved in formalin. *However, the very small numbers of eggs that might be missed in a concentrate obtained from polyvinyl alcohol-preserved stool material are not clinically significant.* If the specimen was collected early in the infection, when egg production was minimal, eggs would be seen in later specimens (if they were submitted because of patient symptoms).

Although dysentery with *T. trichiura* and dysentery with *Entamoeba histolytica* are very similar, whipworm dysentery is usually more chronic, associated with malnutrition, and likely to cause rectal prolapse in children. Recovery and identification of the eggs or protozoan trophozoites would differentiate the two infections. Whipworm infections in heavily infected children may be accompanied by infection with *E. histolytica* and enteropathogenic bacteria. In severe infections, the adult worms are usually visible on the rectal mucosa. Refer to Algorithm 13.3.

KEY POINTS—LABORATORY DIAGNOSIS

Trichuris trichiura

1. Eggs are normally recovered by using the routine ova and parasite examination from the direct wet mount or the concentration procedure wet mount. Egg recovery is generally not as good when concentrations are performed using polyvinyl alcohol-preserved fecal specimens (not commonly performed); formalin or other non-polyvinyl alcohol preservatives are recommended.
2. These eggs should be quantitated on the laboratory report (rare, few, moderate, many), since light infections may not require treatment.
3. Adult worms are very rarely seen; an exception might be during colonoscopy.
4. Most infections are mild; however, in very heavy infections, dysentery may have to be differentiated from that caused by *E. histolytica*.
5. The eggs can usually be identified from the permanent stained smear, but morphology is more easily seen in the wet mount preparations (either direct wet mounts or from concentrated sediment).

Treatment

Several available drugs are very effective, including mebendazole and albendazole. Exact information and dosages are provided in chapter 36. Therapy may not be necessary, depending on the number of eggs recovered in the stool. In investigating the relationship between whipworm infection and cognition in a large group of Jamaican children, one study concluded that albendazole treatment of children with mild to moderate *T. trichiura* infections produced little benefit in cognition if they were adequately nourished; however, children who were undernourished were more likely to benefit (34).

Short-course albendazole (or mebendazole) cures 88 to 95% of infections with *Ascaris*. However, for *Trichuris*, short-course cure rates are low, and patients should receive 3 to 7 days of therapy (16).

Epidemiology and Prevention

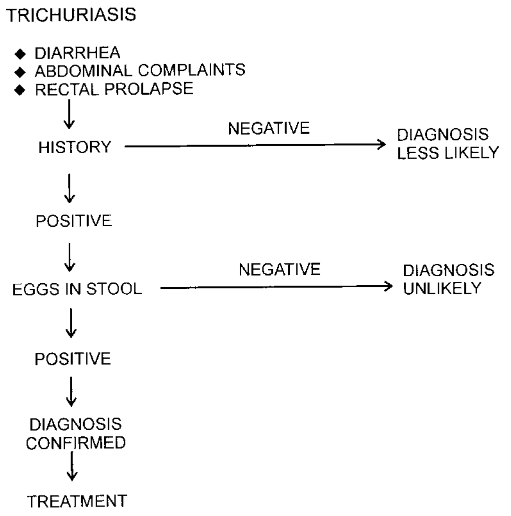
The geographic range of *T. trichiura* is similar to that of *A. lumbricoides*, and the two infections are often found together in the same host. Infection rates are highest in children; the eggs contaminate the soil where the children play, and the children reinfect themselves with eggs transferred from the soil to their mouths. The eggs do not survive dry conditions or intense cold.

Adequate disposal of feces reduces both the number of infections and the worm burdens. This approach is particularly important when one is dealing with children who may be used to defecating on the ground.

Capillaria philippinensis

The first proven case of human infection with *Capillaria philippinensis* occurred in 1963 in a patient from the Philippines who died 3 days after admission to the hospital

Algorithm 13.3 Trichuriasis.
doi:10.1128/9781555819002.ch13.Alg13.3



with a diagnosis of malabsorption syndrome (14). Although the significance was not recognized until 4 years later, *C. philippinensis* eggs were found in the stools and autopsy showed parasitism of the large and small intestines. In 1967, health authorities recognized infections causing severe symptoms and death in adult males, an infection that is now known to be widely distributed in the Northern Luzon area of the Philippines. Since that time, the disease has become widespread in Thailand and cases have been reported in Japan, Taiwan, Iran, and Egypt.

The adult female worms are 2.5 to 4.3 mm long, while males are 2.3 to 3.17 mm long. These worms live in the mucosa of the small bowel, most commonly the jejunum. Human infection is initiated by the ingestion of raw fish; the infective larvae are located in the mucosa of the fish intestine. Specific details of the life cycle are not clearly known; however, there does seem to be an internal autoinfection capability (35). The female worms produce eggs that resemble those of *T. trichiura*. They are thick shelled, with less prominent polar plugs, and the shell is striated (Fig. 13.21). The eggs are somewhat smaller than those of *T. trichiura,* measure 36 to 45 by 20 μm, and require 10 to 14 days in the soil to embryonate and 3 weeks to develop into the infective form in fish.

Life Cycle and Morphology

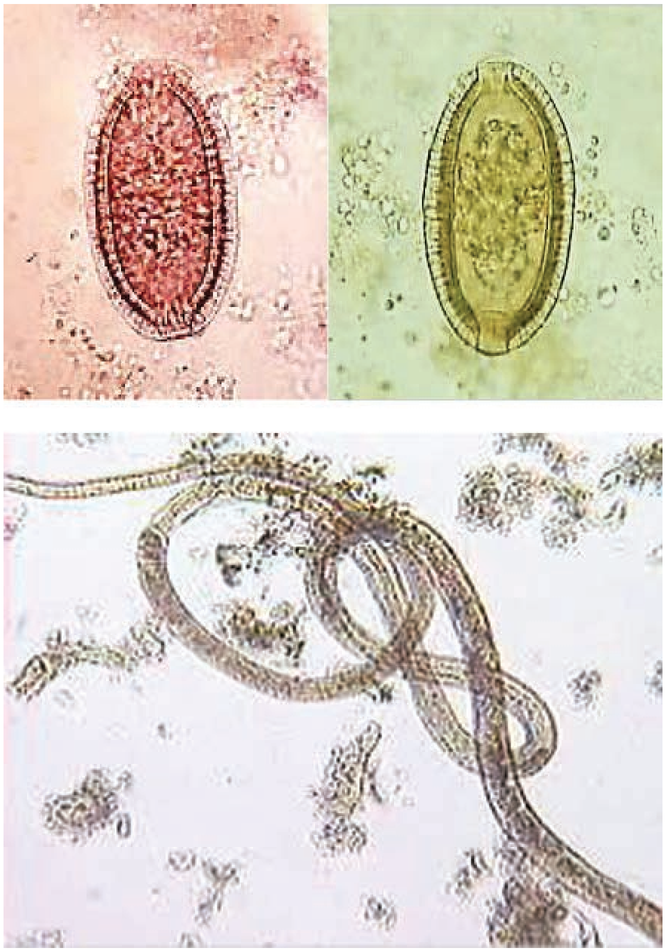
Although the exact mode of transmission is unknown, experimental infection is transmitted through small fish that serve as the intermediate host; often, whole, small fish may be ingested. Development to the infective stage in the fish takes at least 3 weeks. In areas of the Philippines where this infection occurs, people also eat raw shrimp, crabs, and snails. They also tend to defecate in the fields or water where the fish, shrimp, crabs, and snails are obtained, thus completing the life cycle. It has been suggested that defecation from humans to a water resource in the area of endemicity provides an excellent opportunity for *C. philippinensis* eggs to contact and be ingested by naturally susceptible fish.

The worms live burrowed into the mucosa of the small bowel, mainly the jejunum. The adult worms are small. The male has ventrolateral caudal expansions and a very long, smooth spicular sheath. The female has two almost equal parts, with the anterior containing the esophagus and stichosome (esophageal glands) and the posterior containing the intestine and reproductive system. Females produce eggs with thin shells and free larvae, as well as the typical thick-shelled eggs that pass in the stool. All stages of development are seen in the human host, and internal autoinfection is a normal part of the life cycle. Eggs that are passed in stool have been described as "peanut shaped," with flattened bipolar plugs and striated shells, measuring 36 to 45 μm long by 20 μm wide and somewhat resembling *T. trichiura* eggs.

Clinical Disease

Symptoms are related to the worm burden; with large numbers of worms, there may be intestinal malabsorption and fluid loss along with electrolyte and plasma protein imbalance. Most of the abnormality is found in the small intestine, where the wall is thickened and indurated and contains many larval and adult worms. Watery stools are passed (up to eight per day), with fluid loss of several liters. Patients lose weight rapidly and develop muscle wasting, abdominal distention, and edema. Death from pneumonia, heart failure, hypokalemia, or cerebral edema may occur within several weeks to a few months (36). In some cases, patients reported chronic abdominal pain and diarrhea over a period of many months prior to diagnosis. On gastroduodenoscopy and subsequent histology, the jejunal

Figure 13.21 (Upper) *Capillaria philippinensis* eggs. (Lower) Adult worm (courtesy of CDC Public Health Image Library). doi:10.1128/9781555819002.ch13.f21



mucosa revealed flattened villi, crypt proliferation, acute inflammation, and eosinophilic granulomata.

Diagnosis

Diagnosis is based on recovery and identification of the eggs in the stool, which might also contain larvae or adult worms (Fig. 13.21). Knowledge of the geographic range would also provide specific clues to a possible infection. Unfortunately, without some index of suspicion, patients may go for many months with the cause of their symptoms remaining unexplained.

Treatment

Some of the more common therapeutic agents, including mebendazole and albendazole, are effective in treating this infection and result in dramatic improvement in the patient's condition within the first day. Specific drug and dosage information is provided in chapter 36.

Epidemiology and Prevention

Although the life cycle is not completely known, the geographic range is well recognized and continues to expand. Raw infected fish, shrimp, crabs, and snails may serve to transmit the larvae. In areas where this infection is endemic, adequate cooking of all suspect foods should reduce the number of cases. Infections have now been described in the Philippines, Thailand, Japan, Iran, Egypt, Taiwan, and Indonesia.

Hookworms (*Ancylostoma duodenale, Necator americanus*, and *Ancylostoma ceylanicum*)

Although the "Old World" hookworm, *Ancylostoma duodenale,* was mentioned in ancient Egypt in 1600 BCE and in the Persian literature in approximately CE 1000, the first good description was written by Dubini in 1843. Diagnosis was confirmed in the late 1800s by finding eggs in stool, particularly in patients suffering from "miner's anemia." Final delineation of the complete life cycle was completed in 1897 by Arthur Looss from both his accidental self-infection and additional studies on dogs. Although the geographic ranges of *A. duodenale* and the "New World" hookworm *Necator americanus* used to be much more distinct, currently there are many areas of the world where both are found. *N. americanus* was described by Stiles in 1902 as a new species and was soon found to be widely distributed throughout the Western Hemisphere (see below); it is known as the "American hookworm" or the "American murderer."

Hookworm infections are found in moist, warm areas and are responsible for much human disease, although they cause more morbidity than actual mortality. There are distinct morphologic differences between the two most common human hookworms (adult worms); however, the diagnostic stage (egg) is essentially identical for both. Globally, about 900 million people harbor hookworms. In looking at various hookworm isolates, molecular biology-based studies have been very helpful in determining correct classification relationships. Studies of the nucleotide sequences of the second internal transcribed spacer of rDNA of adult worms of *N. americanus* from Togo in Africa, and Sarawak, Malaysia, suggest that there is population variation in the sequence of *N. americanus* or that *N. americanus* from the two countries may represent genetically distinct but morphologically similar species. However, comparison of the sequence differences among other hookworm species supports genetically distinct species (37). As these types of studies continue to examine former classification schemes, we may see generic and species name changes in future publications.

Based on epidemiological, diagnostic, and molecular methods, *Ancylostoma ceylanicum* infection has been found to be common in humans and domestic animals. In a group of 634 human stools from Malaysia, *A. ceylanicum* constituted 12.8% of single infections and 10.6% mixed with *N. americanus*. The probable zoonotic risk of *A. ceylanicum* is a definite public health problem, particularly since the hookworm is highly endemic among domestic animals in these communities with an overall prevalence of 61.9% (38). Recent molecular-based surveys in Asia have shown that *A. ceylanicum* is the second most common hookworm species infecting humans, comprising between 6% and 23% of total hookworm infections. *A. ceylanicum* mimics the clinical picture produced by the anthroponotic hookworms of "ground itch" and moderate to severe abdominal pain in the acute phase. Natural human infections with *A. ceylanicum* have been reported in almost all areas in which the parasite is endemic in dogs and cats (39). A recent case report of an intestinal infestation with *A. ceylanicum* was reported. The patient presented with a 1-month history of diarrhea and a stool positive for occult blood. Her eosinophilia was 50%, and colonoscopy showed multiple moving worms at the terminal ileum. Chronic intestinal infestation in humans may lead to iron deficiency anemia and protein malnutrition (40).

Life Cycle and Morphology

Adult males measure 7 to 11 mm long by 0.4 to 0.5 mm wide. The adult *Ancylostoma* worm tends to be larger than the *Necator* worm. Adult worms are rarely seen, since they remain firmly attached to the intestinal mucosa by means of well-developed mouth parts (teeth in *A. duodenale* and cutting plates in *N. americanus*) (Fig. 13.22).

Infection in humans is acquired through active skin penetration of filariform larvae from the soil (Fig. 13.23).

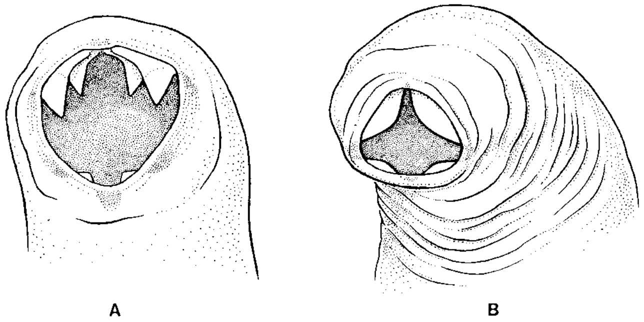


Figure 13.22 (A) *Ancylostoma duodenale* mouth parts (teeth); (B) *Necator americanus* mouth parts (cutting plates). (Illustration by Sharon Belkin; adapted as a composite from a number of photographs, including those in Markell EK et al., *Medical Parasitology*, 7th ed, The WB Saunders Co, Philadelphia, PA, 1992.) doi:10.1128/9781555819002.ch13.f22

The larvae are normally in a motionless, resting position; stimuli for movement include touch, vibration, water currents, heat, light, human breath, and skin extracts such as fatty acids (41). During skin penetration, infective hookworm larvae encounter hyaluronic acid; hookworm hyaluronidase activity has been confirmed and can facilitate passage of the infective larvae through the epidermis and dermis during larval migration (42). The infective larvae of *N. americanus* secrete various classes of proteolytic enzymes with two overall pH optima of 6.5 and 8.5. Since skin penetration is mandatory for the infective larvae, the effect of each of these enzyme classes against macromolecules derived from human skin has been examined. Larval secretions were shown to degrade collagen types I, III, IV, and V, fibronectin, laminin, and elastin. All the skin macromolecules tested were hydrolyzed by aspartyl proteinase activity. Studies of the effect of proteinase inhibitors revealed that larval penetration was significantly inhibited only by pepstatin A, confirming the importance of the aspartyl proteinase activity during skin penetration (43). After skin penetration, the larvae are carried by the venules to the right heart and then to the lungs. Larvae break into the alveoli, migrate via the bronchi until they reach the trachea and pharynx, and are then swallowed, bringing them to the small intestine, where they reside. They attach to the mucosa by using a temporary mouth structure, mature sexually, and finally develop the permanent characteristic mouth structure that they use to attach to the mucosa. The females begin to deposit eggs about 5 months after the initial infection, although the prepatent period may be 6 to 10 months. If mature filariform larvae of *A. duodenale* are swallowed, they can develop into mature worms in the intestine without migrating through the lungs. A *Necator* infection produced by percutaneous exposure of a volunteer to three larvae was monitored by periodic egg counts for 18 years and 4 months (44). Until this report, the longest infection recorded was 15 years.

The eggs are usually in the early cleavage stage when passed in the stool (Fig. 13.24 and 13.25). They are oval with broadly rounded ends and measure approximately 60 μm long by 40 μm wide. They characteristically have a clear space between the developing embryo and the thin eggshell. Although hookworm eggs and eggs of *Trichostrongylus* spp. are similar, specific size differences can be seen in Fig. 13.26. Egg survival and larval development are maximum in moist, shady, warm soil (sandy loam), where larvae hatch from the eggs within 1 to 2 days. The infective filariform larvae develop within 5 to 8 days and may remain viable in the soil for several weeks.

A review by Schad (45) of hookworm developmental biology provides some excellent information on the relationship between arrested development of hookworms and vertical transmission. Epidemiologic evidence supports the

Figure 13.23 Hookworm life cycle. doi:10.1128/9781555819002.ch13.f23

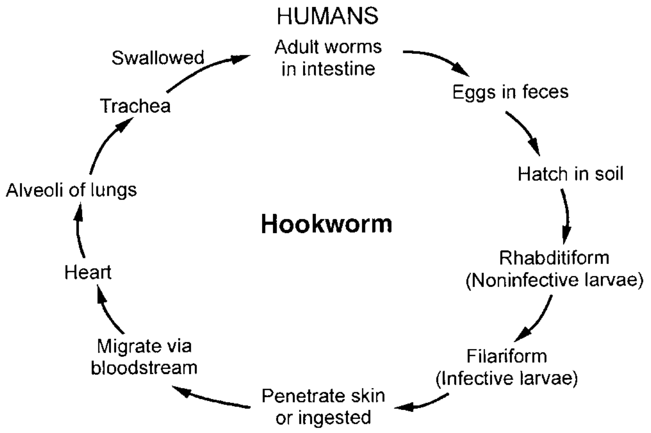
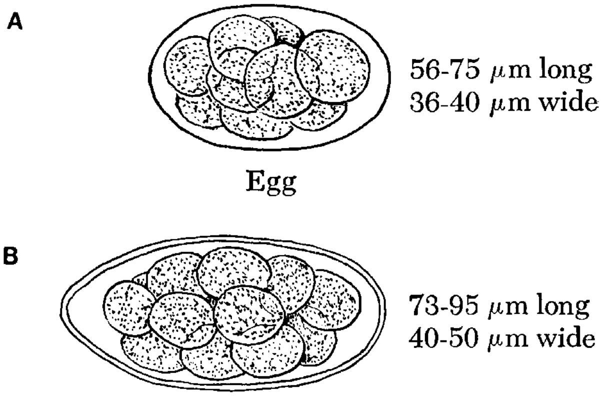


Figure 13.24 (A) Hookworm egg; (B) *Trichostrongylus* egg. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch13.f24



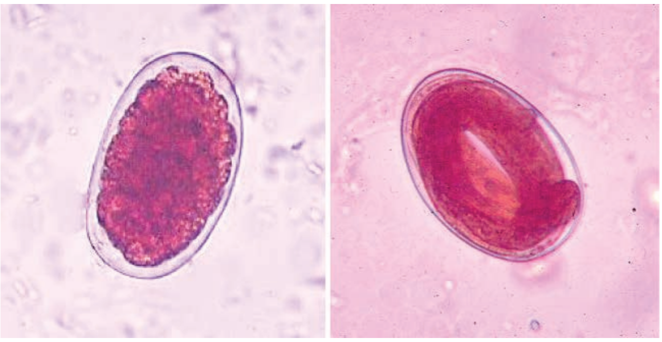


Figure 13.25 (Left) Immature hookworm egg; (right) embryonated hookworm egg.
doi:10.1128/9781555819002.ch13.f25

theory that *A. duodenale* may enter a period of arrested parasitic development for approximately 8 months, as a mechanism allowing the species with the shorter adult life span to survive the dry climatic season in the host. Experimental evidence is based on two self-induced *A. duodenale* infections with very long prepatent periods of 40 weeks (hypobiotic state), about five times the normally expected prepatent period. Clinical evidence involved a group that was exposed to infection and after 11 days left the area where the infection was endemic; hypobiotic larvae were shown to have remained in 8 of 15 patients, with adult and immature worms being expelled after repeated therapy 253 days after the only known exposure. Arrested development is a prerequisite for vertical transmission, and there is mounting evidence that congenital infection can occur (45). Apparently, arrested larvae tend to be highly resistant to most therapeutic agents; control programs that do not take into account this aspect of the life cycle will not achieve optimal results.

Clinical Disease

Initial symptoms after larval penetration of the skin often depend on the number of larvae involved. There may be minimal to severe pruritus and possible secondary infection if the lesions become vesicular and are opened by scratching. The development of vesicles from the erythematous papular rash is called ground itch.

Any pneumonitis due to migrating larvae depends on the number of larvae present. These larvae do not cause the same level of sensitization seen with *Ascaris* or *Strongyloides* infection.

Symptoms from the intestinal phase of the infection are caused by (i) necrosis of the intestinal tissue within the adult worm mouth and (ii) blood loss by direct ingestion of blood by the worms and continued blood loss from the original attachment site, possibly as a result of anticoagulant secreted by the worm (Fig. 13.27).

Patients with acute infections involving many worms may experience fatigue, nausea, vomiting, abdominal pain, diarrhea with black to red stools (depending on the level of blood loss), weakness, and pallor. As in many other parasitic infections, heavy worm burdens in young children may have serious sequelae, including death. During this acute intestinal phase, there is an increased peripheral eosinophilia.

In chronic infections, the main symptom is iron deficiency anemia (microcytic, hypochromic) with pallor, edema of the face and feet, listlessness, and hemoglobin

Figure 13.26 (Left) *Trichostrongylus orientalis*, immature egg; (right) hookworm, immature egg.
doi:10.1128/9781555819002.ch13.f26

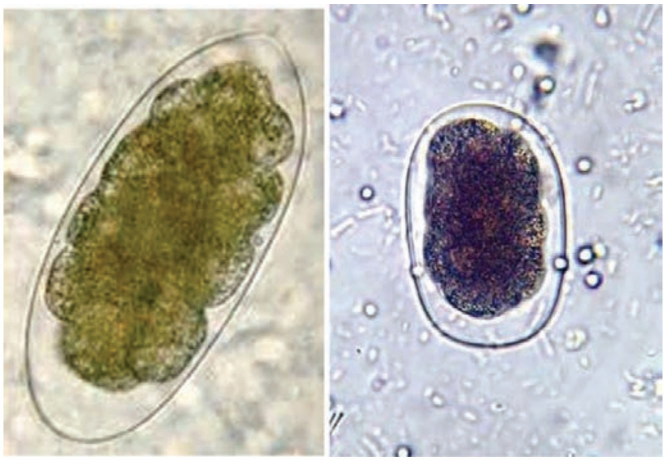
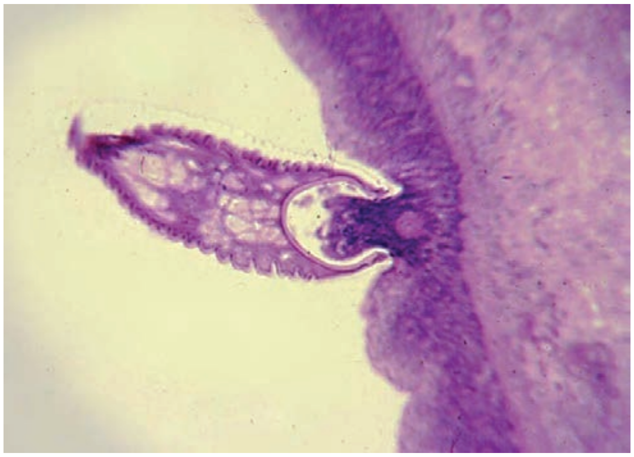


Figure 13.27 Hookworm, section of a worm attached to the mucosa. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch13.f27



levels of 5 g/dl or less. There may be cardiomegaly and both mental and physical retardation.

The hookworms cause gastrointestinal blood loss, and in clinical studies significant blood losses have been reported with *A. duodenale*. However, there has been no evidence that endemic *A. duodenale* infection has a greater impact than *N. americanus* infection on the iron status of populations. In a sample of 525 schoolchildren in Tanzania, the degree of anemia and iron deficiency associated with the two hookworm species was compared at the individual and community school levels. Hemoglobin and ferritin concentrations decreased with increasing proportions of *A. duodenale*. In children infected with only *N. americanus* larvae, the prevalence of anemia was 60.5% and the prevalence of low ferritin levels was 33.1%, while in children with at least 50% *A. duodenale* larvae, the respective prevalences were 80.6 and 58.9%. When children were grouped by prevalence of *A. duodenale* at the school level, children from high-prevalence (≥20%) schools had significantly worse iron deficiency and anemia than did children from low-prevalence schools. Since the species of hookworm being transmitted within a community influences the burden of iron deficiency anemia in the community, this factor should be considered in prioritizing and planning programs for hookworm and anemia control (46). It is also well documented that there is a relationship between the hookworm worm burden and anemia during pregnancy; women with moderate or heavy hookworm infections were more likely to suffer from anemia than were women having light infections. These findings support routine therapy within prenatal care programs in areas where infection is highly endemic (47).

Egg counts of 5/mg of stool are rarely clinically significant, counts of more than 20/mg are usually associated with symptoms, and counts of 50/mg or more represent very heavy worm burdens.

Immunology and Host Resistance

There is a mixed Th1-Th2 response in hookworm infection with subsequent immunosuppression of gamma interferon responses. There is also evidence for protective immunity in hookworm infection, including antilarval IL-5- and IgE-dependent mechanisms (48). Antihookworm antibodies show cross-reactivity with *A. lumbricoides* and *Schistosoma mansoni,* while IgG4 or IgE responses tend to be more specific. The levels of polyclonal IgG in serum and intestinal IgG, IgM, and IgE in the intestines are also elevated, while intestinal IgA levels are reduced.

Eosinophilia is common, usually develops 25 to 35 days after exposure, and peaks about a month (*N. americanus*) to 2 months (*A. duodenale*) later. Proliferative lymphocyte responses tend to be variable, while cytokine results indicate that hookworm infection produces a mixed Th1-Th2 response. There is significant production of Th1 (gamma interferon and IL-12) and Th2 (IL-4, IL-5, and IL-13) cytokines. There are also high levels of IL-10 and tumor necrosis factor alpha.

Hookworm-mediated immunosuppression has relevant implications for vaccine development. Unless preexisting infections are cleared by therapy, vaccine-induced immunity may be susceptible to immunosuppression. Also, if the vaccine is only partially effective, vaccine recipients may be susceptible to reinfection, which might further suppress vaccine efficacy. Careful selection of vaccine candidates may prevent this problem. Currently, the Na-APR-1M74 protease is being examined as an antigen component for the human hookworm vaccine (49).

There is evidence for a protective role of helminth infection against asthma and malaria; however, helminth infection may increase susceptibility to HIV/AIDS or tuberculosis. People with hookworm infection are also more likely to be infected with *A. lumbricoides* and *T. trichiura,* findings that can only partially be explained by overlapping areas of endemic infection and potential exposure.

Diagnosis

Definitive diagnosis of hookworm infection depends on demonstration of the presence of eggs in stool, especially since the symptoms cannot be differentiated from malnutrition (Fig. 13.25). The eggs are best seen in the direct smear or the concentration sediment; they are distorted on the permanent stained smear.

Note If the stool specimen is stored at room temperature (no preservative) for more than 24 h, the larvae continue to mature and hatch. These larvae must be differentiated from *Strongyloides* larvae, since therapy may be different for the two infections.

Many infections seen in the United States are not severe, and eggs in the stool may represent an incidental finding. Radiographic findings include intestinal hypermotility, proximal jejunal dilatation, and coarsening of the mucosal folds (14). Refer to Algorithm 13.4.

Chronic intestinal infestation in humans with *A. ceylanicum* may lead to iron deficiency anemia and protein malnutrition (40). Diagnosis could be made from finding the worms; colonoscopy could be helpful (Fig. 13.28).

In one study examining stools for helminth eggs by the Kato-Katz method and by a modified Harada-Mori nematode larval culture method, there was an 81.8% agreement between the two methods for the detection of hookworm infection, an infection that was endemic in the study population and highly prevalent in pregnant women in the area (50).

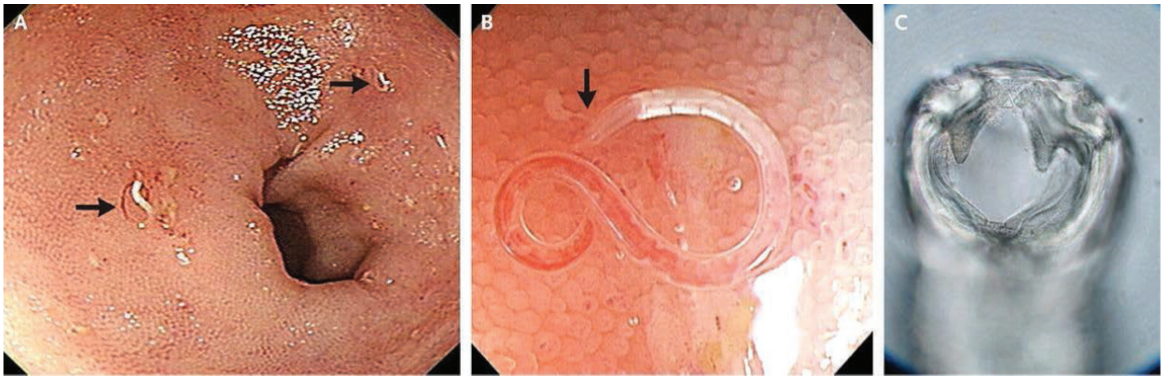


Figure 13.28 *Ancylostoma ceylancum.* (Left) Worms seen at colonoscopy (arrow); (middle) worm with head buried in mucosa and engulfing blood (arrow); (right) hookworm mouth parts. (Courtesy of Hsu YC, Lin JT, *N Engl J Med* **366:**e20, 2012.) doi:10.1128/9781555819002.ch13.f28

KEY POINTS—LABORATORY DIAGNOSIS

Hookworms *(Ancylostoma duodenale*, *Necator americanus*, and *Ancylostoma ceylanicum)*

1. Eggs are normally passed in the stool in the unembryonated state (usually about an 8- to 32-cell stage of development). Typically these have a thin shell and clear space between the developing embryo and the shell.
2. If the stool remains unpreserved for over 24 h, the eggs may continue to develop and the larvae may hatch. These larvae must be differentiated from those of *S. stercoralis*, since therapies for the two infections are different.
3. The eggs are normally distorted on the permanent stained smear, and morphology is more easily seen in wet preparations.
4. Adult worms could be seen at colonoscopy.

Treatment

Several effective therapeutic agents, including mebendazole, pyrantel pamoate, and albendazole, are available in the United States. Additional studies support the data showing that, despite its relatively low cost and wide availability, pyrantel pamoate at a single-dose rate of 10 mg/kg of body weight is not effective against *A. duodenale* and should not be considered a drug of choice at this dose rate. In contrast, albendazole (at a single 400-mg dose) cleared hookworm infections completely (51). Confirmatory studies also indicate that in the southern region of Mali, single-dose treatment with mebendazole is not an effective treatment for hookworm infections and that, despite its relatively low cost and wide availability, mebendazole should not be considered a drug of choice in the mass treatment of hookworm infections (52). See chapter 36 for specific drug and dosage recommendations.

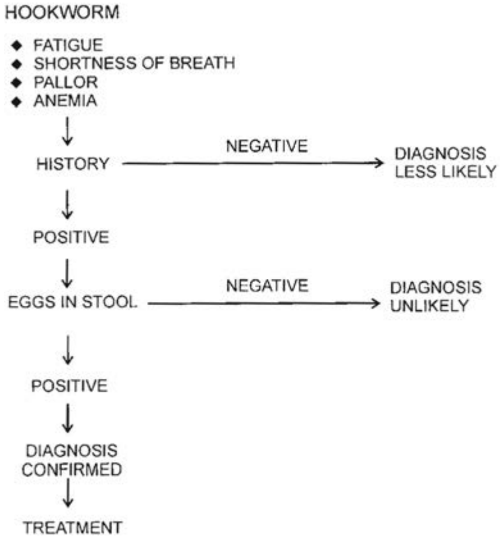
Adequate nutrition can prevent and overcome disease symptoms but not the infection itself. Apparently, adequate absorption of iron and nutrients is not possible in heavy infections, even with extensive dietary supplements.

Epidemiology and Prevention

Sanitary disposal of feces is the primary means of infection control. This is sometimes difficult in poor rural communities where sanitary facilities are minimal or absent. An overall prevention approach also requires educational programs. The wearing of shoes and the use of soil sterilization have not proven to be practical.

Several factors influence hookworm prevalence: infection in the human population, defecation onto the soil, suitable environmental conditions, and human contact with the infective larvae in soil. Environmental conditions

Algorithm 13.4 Hookworm.
doi:10.1128/9781555819002.ch13.Alg13.4



include temperature, rainfall pattern, and the presence of open, sandy soil.

A significant increase in the prevalence of hookworm infection has been seen in an area of Haiti where intestinal parasites are common but hookworm had not been common. Changing environmental conditions, specifically deforestation and subsequent silting of a local river, have caused periodic flooding with deposition of a layer of sandy loam topsoil and increased soil moisture. These conditions, all of which are conducive to hookworm transmission, have allowed hookworm to reemerge as an important human pathogen in this area. This example emphasizes the value of longitudinal surveillance data for monitoring disease prevalence. Shifts in the prevalence of infectious diseases can be caused by environmental changes, including planned human activity, or can be an indirect consequence of political strife, and these factors should always be considered when changes in infectious disease patterns are detected.

A. duodenale (Old World hookworm) is found primarily in southern Europe, the north coast of Africa, northern India, northern China, and Japan. *N. americanus* (New World hookworm) is found throughout the southern United States, the Caribbean, Central America, northern South America, central and southern Africa, southern Asia, Melanesia, and Polynesia. In some areas such as northern Ghana, both types of hookworms are present; mixed infections have been confirmed using PCR; overlapping geographic areas are becoming more common (53).

Trichostrongylus spp.

Trichostrongylus nematodes are commonly found in herbivores throughout the world. Various species have been found in humans, and some are more clinically important than others. Throughout the world, there are certain areas of endemic infection where the percentage of positive patients is substantial, and at least 10 species have been found to be human parasites. Human infections tend to be prevalent in Iran, while sporadic infections have been reported from many countries.

Life Cycle and Morphology

Trichostrongylus spp. are small worms, similar to hookworms, and live embedded in the mucosa of the small intestine. Unlike the adult hookworms, the adult worms have no distinct buccal capsule with special mouth parts (teeth or cutting plates).

Infection in humans is acquired through ingestion of the infective larvae contaminating plant material. After reaching the small intestine, the larvae mature in 3 to 4 weeks without any migratory pathway through the lungs. The eggs are very similar to those of hookworms, being oval and somewhat longer, with the ends being more pointed than in hookworm eggs (Fig. 13.26 and 13.29). The eggs may hatch within 24 h under favorable conditions (warm, moist soil) and develop into infective larvae after about 60 h.

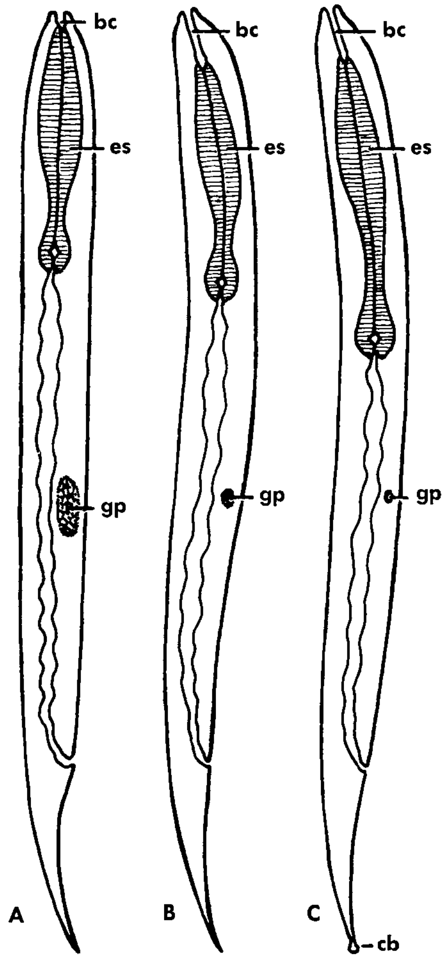


Figure 13.29 Rhabditiform larvae. (A) *Strongyloides stercoralis*; (B) hookworm; (C) *Trichostrongylus* sp. Abbreviations: bc, buccal cavity; es, esophagus; gp, genital primordium; cb, beadlike swelling of caudal tip. (Illustration by Nobuko Kitamura; from Garcia LS, *in* Finegold SM, Baron EJ, *Diagnostic Microbiology*, 7th ed, The CV Mosby Co, St. Louis, MO, 1986.)
doi:10.1128/9781555819002.ch13.f29

Clinical Disease

Symptoms are related to the worm burden and damage to the intestinal mucosa. Hemorrhage and desquamation may occur (similar to findings in hookworm infection); however, symptoms are usually not clinically significant unless several hundred worms are present. Patients present with epigastric pain, diarrhea, anorexia, nausea, dizziness, and generalized fatigue or malaise; eosinophilia is usually present. Heavy worm loads may lead to the development of anemia and cholecystitis, as the worms enter the biliary tract. A *Trichostrongylus* infestation has been seen masquerading as conditioning toxicity of the gut in a bone marrow transplant recipient, another reason to consider all possibilities in immunocompromised patients (54).

Diagnosis

The definitive diagnosis can be made by identification of eggs in the stool. Hatched larvae can also be differentiated from those of hookworms and *S. stercoralis* (Fig. 13.29).

Treatment

Several anthelmintic agents are available, including pyrantel pamoate, mebendazole, and albendazole, some of which are also used for the other nematode infections. Specific drug and dosage information is presented in chapter 36.

Epidemiology and Prevention

Since the infection is found in herbivores throughout the world, any grazing areas are constantly being reinfected. In some areas of the world where animal dung is formed into solid masses, dried, and burned for fuel, this practice may be important in the transmission of animal *Trichostrongylus* to humans. Frequently, animal manure is used as an organic fertilizer, thus contaminating greens and other vegetables used for human consumption. The main preventive measures are adequate cleaning and cooking of all vegetables prior to eating.

Based on newer epidemiologic reports, eight human cases in Thailand and the Lao People's Democratic Republic have been found to be infected by *Trichostrongylus colubriformis* and *T. axei*. These species were identified and confirmed using molecular techniques and represent the first evidence for endemicity of these two species in these areas (55).

Strongyloides spp.

Strongyloides stercoralis was first found in 1876 in the feces of French soldiers with diarrhea who were returning from Indochina. A series of workers dealt with the classification, life cycle differentiation, and possible pathogenesis of this worm during the early 1900s. Early work led to demonstration of the internal autoinfective portion of the life cycle, which has become extremely important, particularly when one is working with immunocompromised patients. *S. stercoralis* is most commonly found in warm areas but can survive in colder climates. The infection is most often found to overlap the geographic range of hookworm infections and has been reported as the most commonly diagnosed helminth infection at the University of Kentucky Medical School. It is very important to consider this infection in military personnel and travelers who, many years earlier, were in an area where the infection is endemic. More than 30 to 40 years after acquisition of the original infection, persistent, undiagnosed disease can be found in these individuals. If for any reason they become immunocompromised, the result can be disseminated disease leading to the hyperinfection syndrome and death.

Life Cycle and Morphology

The life cycle of *S. stercoralis* is one of the more complex cycles and can be seen in Fig. 13.30. Human infection is acquired by skin penetration of the filariform larvae (infective larvae) from the soil. These larvae are long and slender (up to 630 μm long by 16 μm wide) and may remain viable in soil or water for several days. After penetration of the skin, the larvae are carried via the cutaneous blood vessels to the lungs, where they break out of the pulmonary capillaries into the alveoli. They then migrate via the respiratory tree to the trachea and pharynx, are swallowed, and enter the mucosa in the duodenum and upper jejunum. Development of the female worms to adults usually takes about 2 weeks, after which the females begin egg production by parthenogenesis. The eggs are oval and thin shelled, and they measure 50 to 58 μm long by 30 to 34 μm wide (generally a bit smaller than hookworm eggs). They hatch, and the rhabditiform larvae (noninfective larvae) pass out of the intestinal tract in the feces onto the soil where they develop into free-living male and female worms, eventually producing infective filariform larvae (egg, noninfective larvae, and infective larvae). In temperate climates, the free-living male and female worms do not develop; however, the rhabditiform larvae that pass out in the stool develop into the filariform (infective) larvae, which are then ready to infect the next host through skin penetration.

S. fuelleborni is also transmitted primarily by skin contact. However, investigations into the high prevalence of *S. fuelleborni* in African children have led to the postpartum identification of larvae in breast milk. In Papua New Guinea, high rates of infection (up to 65%) in children younger than 3 months suggest that transmammary infection might also occur in this area, although the parasite has not been identified in breast milk. Also, *S. fuelleborni* has been identified as the causative agent of "swollen belly syndrome," a fulminant and fatal enteritis that has been well documented in infants younger than 6 months in areas of highly endemic infection in Papua New Guinea. It has been suggested that transmammary infection followed by early external autoinfection from dirty diapers may result in rapid multiplication of worms in these young children (56).

In situations in which autoinfection occurs, some of the rhabditiform larvae that are within the intestine de-

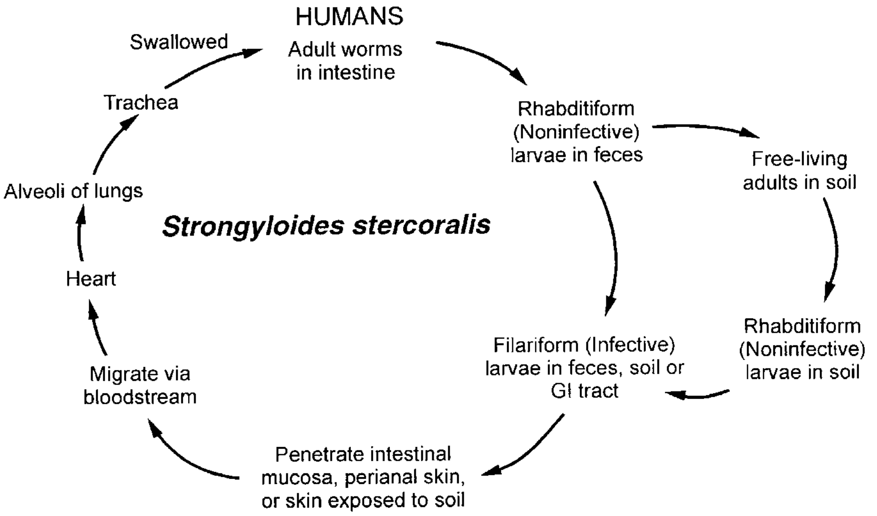

HUMANS
Adult worms in intestine
Rhabditiform (Noninfective) larvae in feces
Free-living adults in soil
Rhabditiform (Noninfective) larvae in soil
Filariform (Infective) larvae in feces, soil or GI tract
Penetrate intestinal mucosa, perianal skin, or skin exposed to soil
Migrate via bloodstream
Heart
Alveoli of lungs
Trachea
Swallowed
Strongyloides stercoralis

Figure 13.30 *Strongyloides stercoralis* life cycle. doi:10.1128/9781555819002.ch13.f30

velop into the filariform larvae while passing through the bowel. These larvae can then reinfect the host by (i) invading the intestinal mucosa, traveling via the portal system to the lungs, and returning to the intestine, or (ii) being passed out in the feces and penetrating the host on reaching the perianal or perineal skin.

The rhabditiform larvae that normally pass out in the stool measure up to 380 μm long by 20 μm wide, with a muscular esophagus (club-shaped anterior, then a restriction, and a posterior bulb) (Fig. 13.31). There is a genital primordium packet of cells, which is fairly obvious and can be seen about two-thirds of the way back from the anterior end (Fig. 13.32). One of the key morphologic differences between these larvae and those of hookworm is the length of the mouth opening (buccal capsule). The opening in the rhabditiform larvae of *S. stercoralis* is ex-

Figure 13.31 Buccal cavity (rhabditiform larvae). (Left) *Strongyloides stercoralis*; (right) hookworm. doi:10.1128/9781555819002.ch13.f31

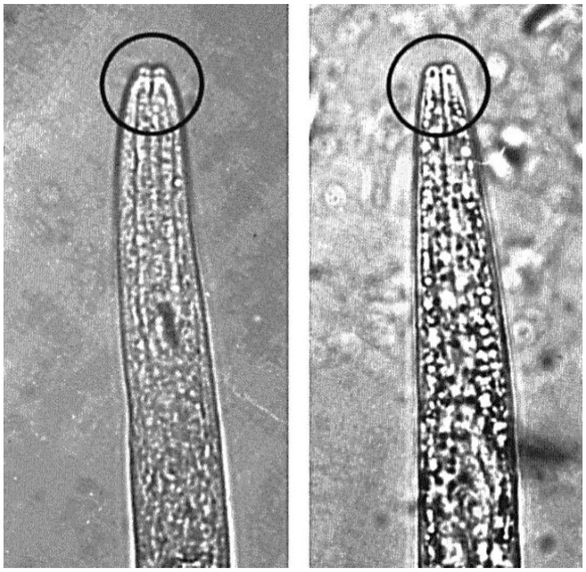
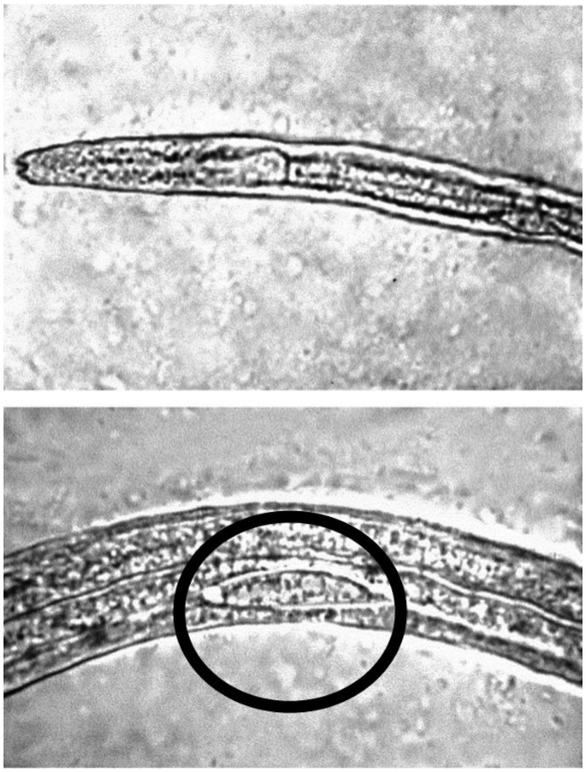


Figure 13.32 (Upper) *Strongyloides stercoralis* rhabditiform larva; note the packet of genital primordial cells toward the right side of the image. (Lower) *S. stercoralis* rhabditiform larva; note the packet of genital primordial cells in the middle of the field (enlarged) (circle). doi:10.1128/9781555819002.ch13.f32



tremely short (only a few micrometers), while the mouth opening in hookworm rhabditiform larvae is approximately three times as long (Fig. 13.29 and 13.31). These differences can be seen by examining the larvae under the microscope with the low (10×) or high dry (40×) objectives.

Strongyloides spp. have the ability to switch between alternative free-living developmental pathways in response to changing environmental conditions. Anterior chemosensory neurons (amphidial neurons) are thought to respond to environmental cues and, via signal transduction pathways, control the direction of larval development. Larvae that pass to the exterior may develop directly to infectivity by the homogonic route or may develop by the heterogonic route to establish a population of soil-dwelling dioecious adult worms. The infective larvae produced by either route are environmentally resistant, developmentally arrested worms that can develop further only after entering the host. Studies confirm that neurons homologous to the two classes of amphidial neurons (ASF and ASI) do occur in *S. stercoralis* and that they control the direction of free-living development in this species (57). These neurons are probable homologues of neurons ADF (=ASF) and ASI in *Caenorhabditis elegans*, thus control of development at the cellular level is probably conserved among divergent taxa of nematodes. Apparently, the free-living cycle develops most frequently in tropical climates with moist soil while the parasitic life cycle may predominate in more temperate climates.

Review of the Life Cycle. The life cycle of *S. stercoralis* contains the following phases, some or all of which may be present, depending on environmental conditions:

1. Free-living forms in the soil that can produce infective larvae (capable of human skin penetration, common in the moist, warm tropics).
2. Noninfective rhabditiform larvae that pass in the stool and transform into infective filariform larvae while in the soil (capable of human skin penetration, common in temperate zones and responsible for most human infections).
3. Autoinfection, which involves development of filariform larvae in or on the body (may account for continued infection 30 to 40 years after the host has left the original area where the infection is endemic) (58).
4. Information from animal studies also suggests that chronic infections result from (i) periodic reactivation of third-stage larvae from a reservoir of dormant parasites outside the gastrointestinal tract or (ii) the periodic rejuvenation of postreproductive female worms remaining from a previous infection, lodged in the mucosal crypts, particularly if the host was treated with corticosteroids (59).

Comments on the Life Cycle. Although it is generally accepted that the life cycle presented above is correct, the skin-penetrating larvae travel from the skin to the intestine via an obligatory route that includes the blood, lungs, trachea, and upper gastrointestinal tract (the standard pulmonary route). It is also assumed that, following autoinfection, the larvae follow the same route to return to the duodenum, where they mature. However, review information and data from Schad et al. (60) provide evidence to indicate that the pulmonary route is just one of several possible pathways to the duodenum, regardless of whether the larvae penetrated the skin or the intestine. Direct sampling of the larvae within the trachea gave an estimate of the number of larvae within the duodenum, presumably transmitted by the pulmonary route, that was insufficient to account for the number actually found there. In spite of some caveats, these studies definitely challenge the current accepted understanding of the life cycle. The pulmonary migration route for all skin-penetrating nematodes may not be as universally applicable as was once thought (60).

Clinical Disease

The pathologic changes associated with strongyloidiasis can vary both in severity and in the areas of the body involved. Some individuals may remain totally asymptomatic, with the only abnormal clinical finding being a peripheral eosinophilia (61). Immunosuppressive therapy, viral infection-associated immunodeficiency, alcoholism, and diabetes mellitus have been identified as risk factors for reactivation of chronic inapparent strongyloidiasis. However, in many cases, review of hematology data suggests the low sensitivity of hypereosinophilia as a marker for this infection.

Considering the life cycle, the three areas of primary involvement would be the skin (cutaneous), the lungs (pulmonary), and the intestinal mucosa (intestinal).

Cutaneous. Initial skin penetration usually causes very little reaction, although there may be some pruritus and erythema if the number of penetrating larvae is large. In some patients, the reaction at the site of larval penetration may last several weeks. With repeated infections, the patient may mount an allergic response that prevents the parasite from completing the life cycle. The larvae may be limited to skin migration (larva migrans). The term "larva currens" (racing larva) was proposed in 1958 and is now generally accepted for cases of strongyloidiasis in which there is one or more rapidly progressing linear urticarial tracks starting near the anus. There is speculation that some of these cases may involve larvae of other species of *Strongyloides*. These tracks may progress as fast as 10 cm/h, with an intermittent movement, usually on the thighs. Onset is sudden, and the lesions may disappear within 12

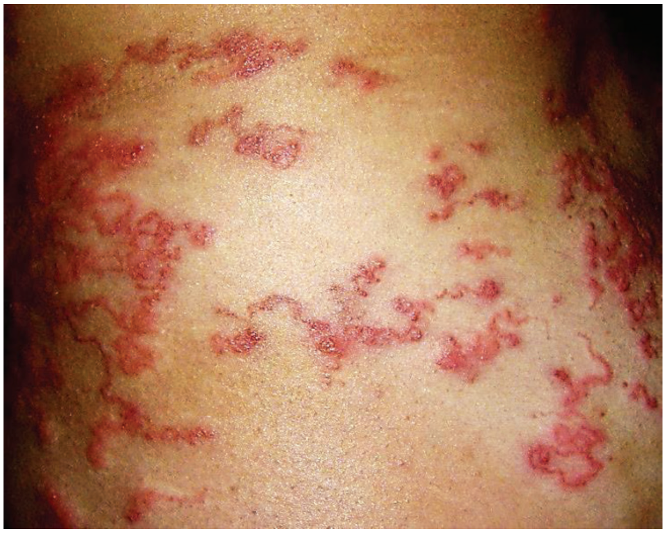


Figure 13.33 *Strongyloides stercoralis* larva currens. (Courtesy of CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch13.f33

to 18 h (14) (Fig. 13.33). Pruritus and eruption disappear when larvae enter blood vessels and migrate to intestinal mucosa.

Pulmonary. Larval migration through the lungs may stimulate symptoms, depending on how many larvae are present and the intensity of the host immune response. Some patients may be asymptomatic, while others may present with pneumonia. With a heavy infective dose or in the hyperinfection syndrome, individuals often develop cough, shortness of breath, wheezing, fever, and transient pulmonary infiltrates (Loeffler's syndrome). There have been cases reported in which the larvae were found in the sputum during this larval migration (Fig. 13.34).

Intestinal. Although the timing may vary from person to person, gastrointestinal symptoms such as diarrhea, constipation, anorexia, and abdominal pain usually begin about 2 weeks after infection, with larvae being detectable in the stool after 3 to 4 weeks. In heavy infections, the intestinal mucosa may be severely damaged, with sloughing of tissue, although this type of damage is unusual (Fig. 13.35). Parasitized individuals may show villous atrophy and crypt hyperplasia. There is infiltration of the lamina propria by IgA-positive plasma cells and of the epithelium by intraepithelial lymphocytes. Infection is also associated with increased expression of secretory component and decreased expression of HLA-DR in epithelial cells. These changes tend to correlate with the degree of clinical severity. In a group of immunocompetent patients with chronic *S. stercoralis* infection, no abnormality of mucosal structure and no increase in the levels of nonspecific inflammatory cells were found. Likewise, there was no increase in the numbers of mucosal T cells or macrophages (62). The symptoms may mimic a peptic ulcer, with abdominal pain which may be localized in the right upper quadrant. Radiographic findings may mimic those of Crohn's disease of the proximal small intestine. In immunocompetent patients, there is leukocytosis with peripheral eosinophilia of 50 to 75%, whereas in patients with chronic cases, the eosinophilia may be much lower. Some of these chronic infections have lasted for over 30 years as a result of the autoinfective capability of the larvae. One case of chronic strongyloidiasis persisted for approximately 65 years.

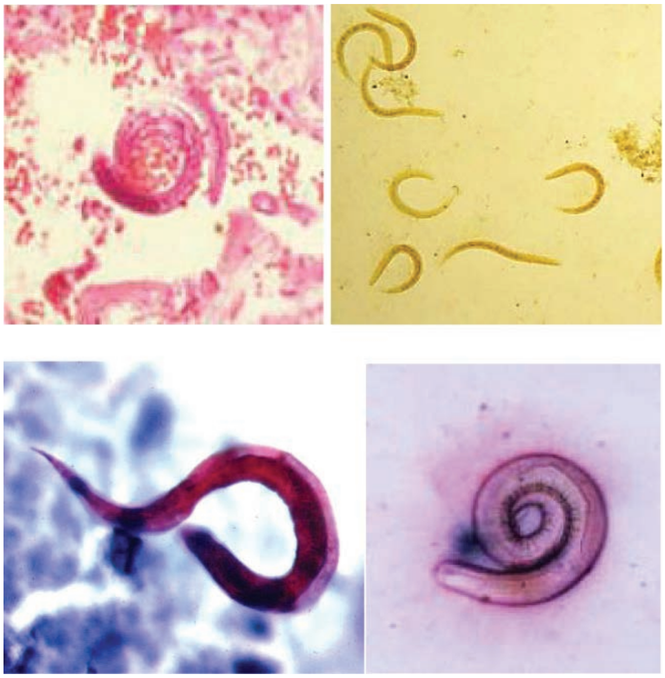


Figure 13.34 (Upper left) *Strongyloides* larvae in lung tissue (routine hematoxylin and eosin stain); (right) *Strongyloides* larvae in direct wet mount of stool (heavy infection). (Lower left) *Strongyloides* larva in stool (Wheatley's trichrome, routine ova and parasite stain); (lower right) *Strongyloides* larva in Gram stain.
doi:10.1128/9781555819002.ch13.f34

Chronic Infection. Chronic infections are usually asymptomatic; however, there may be symptoms that are unrelated to autoinfection. These symptoms include intermittent vomiting, diarrhea, constipation, and loud gurgling noises produced by movement of gas, fluid, or both in the gastrointestinal tract. Anal pruritus, urticaria and larva currens rashes, recurrent asthma, and nephrotic syndrome have also been associated with chronic disease. With development of the hyperinfection syndrome, intestinal obstruction, ileus, and gastrointestinal bleeding can also occur.

Hyperinfection Syndrome. The hyperinfection syndrome has been linked to immunosuppression. Conditions that im-

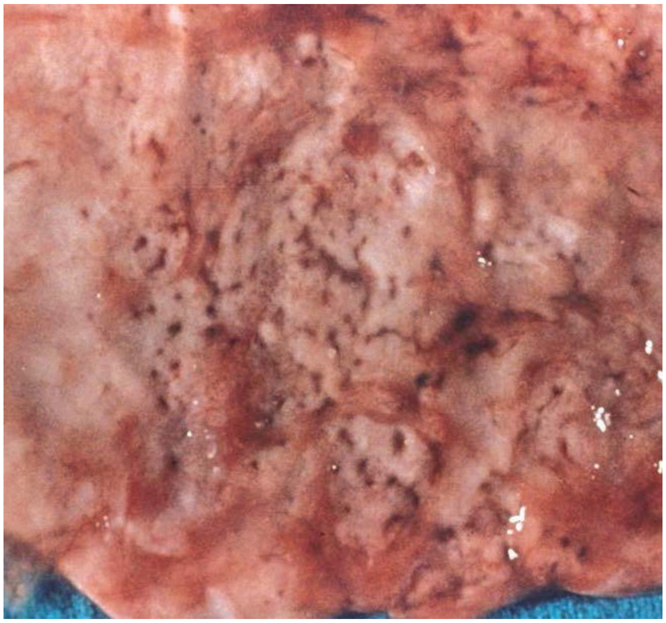


Figure 13.35 *Strongyloides stercoralis*, ulcerations seen in the stomach mucosa. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch13.f35

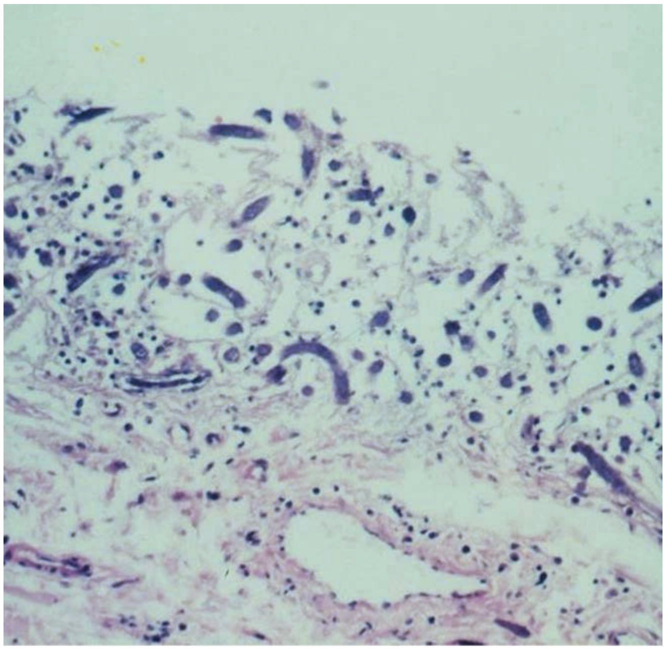


Figure 13.36 *Strongyloides stercoralis*, hyperinfection in the colon of an immunosuppressed patient. (From *A Pictorial Presentation of Parasites*, a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch13.f36

pact on cell-mediated immunity have been identified as risk factors for the development of this syndrome. The two conditions that are most frequently recognized as predisposing factors for the development of the hyperinfection syndrome are corticosteroid use and human T-lymphotropic virus type 1 (HTLV-1) infection.

Autoinfection and other mechanisms are responsible for long-term infections that persist years after the person has left the area where the infection is endemic. The parasite and host reach a status quo so that neither suffers any serious damage. If for any reason this equilibrium is disturbed and the individual becomes immunosuppressed, the infection proliferates, with large numbers of larvae being produced and found in every tissue of the body, including the gastric mucosa (Fig. 13.35). Several conditions, including increased use of immunosuppressive therapy, predispose an individual to the hyperinfection syndrome (63). However, the distinction between autoinfection and hyperinfection tends to be quantitative rather than being specifically defined (Fig. 13.36; Table 13.4).

In contrast to the usual *Strongyloides* hyperinfection syndrome seen in patients with some forms of immunodeficiency, in which small intestinal and pulmonary symptoms are seen, in one group of patients only a characteristic transmural eosinophilic granulomatous inflammation, affecting mostly the colonic wall and clinically mimicking ulcerative colitis or Crohn's disease, has been found. This eosinophilic granulomatous enterocolitis apparently results from a florid inflammatory response by eosinophils, histiocytes, and giant cells, with formation of granulomas that destroy the larvae entering the colon (64). This morphologic picture differs from the well-described hyperinfection syndrome, in which the majority of the larvae pass through the colonic wall to complete the life cycle, with only a few larvae destroyed in the colon.

Transplantation. *Strongyloides* infections in solid organ and bone marrow transplant recipients are thought to be caused by reactivation of chronic infection after initiation of immunosuppressive therapy, which can lead to hyperinfection or disseminated disease. The American Society for Transplantation's guidelines are in place to screen solid organ transplant recipients, but not donors, to assess the risk for reactivation of chronic infection in those from areas in which *Strongyloides* is endemic. However, donor-derived infection might be more common than previously believed, and screening of donors from areas of endemicity might help to protect organ recipients. Rapid communication among transplant centers with patients who received organs from a single donor also is essential (65). *Strongyloides* hyperinfection can happen any time after transplantation; however, this condition usually occurs within the first 3 months during times of increased immunosuppression.

Strongyloides hyperinfection and dissemination are recognized complications in kidney transplant recipients;

TABLE 13.4 Strongyloidiasis hyperinfection syndrome[a]

Body site	Symptoms	Diagnosis	Comments
Gastrointestinal tract	Crampy or bloating abdominal pain; watery diarrhea, constipation, anorexia, weight loss, difficulty swallowing, sore throat, nausea, vomiting, gastrointestinal bleeding; ileus and small bowel obstruction; diffuse abdominal tenderness, hypoactive bowel sounds; hypoalbuminemia; hypokalemia	Direct stool exam shows numerous rhabditiform and filariform larvae; occasionally adult worms and eggs; mucosal ulceration common in small intestine.	Symptoms are very common, but nonspecific; some patients are asymptomatic.
Cardiopulmonary system	Cough, wheezing, choking sensation, hoarseness, chest pain (pleuritic); hemoptysis; palpitations, atrial fibrilliation, dyspnea, respiratory collapse (rare)	Direct sputum exam shows rhabditiform and filariform larvae; occasionally eggs; chest X rays show bilateral or focal interstitial infiltrates.	If condition goes undiagnosed and steroids are given, symptoms may improve temporarily.
Skin and connective tissue	Pruritic linear streaks on lower trunk, thighs, and buttocks; petechial and purpuric rashes; evidence of vasculitis (larva currens)	Larvae can be seen in skin biopsy	Associated Gram-negative bacterial sepsis and/or disseminated intravascular coagulation may be present.
Central nervous system	Meningeal symptoms; hyponatremia and meningitis; signs of aseptic meningitis in spinal fluid (pleocytosis, elevated protein, normal glucose, negative cultures); Gram-negative bacterial infection	Larvae recovered in spinal fluid	Typical Gram-negative bacteria isolated include *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Streptococcus bovis*; immunosuppressed patients may develop *Candida* infections.
Other infected organs	Liver: elevated liver enzymes	Larvae may be seen in various tissues	Other body sites include mesenteric lymph nodes, gallbladder, diaphragm, heart, pancreas, muscle, kidneys, and ovaries.

[a] Adapted from reference 63.

however, strongyloidiasis in renal transplant recipients receiving cyclosporin A has not been described, nor have any cases of strongyloidiasis complicating heart, liver, lung, or pancreas transplantation been published prior to this case. One possible explanation for this observation is the antiparasitic activity of cyclosporin A found in animal studies. In one renal transplant patient, *Strongyloides* hyperinfection occurred immediately after cyclosporin A treatment was discontinued. Approximately 44 days after transplantation, ongoing cellular rejection was confirmed via renal biopsy and cyclosporin A treatment was discontinued according to protocol. His earlier history was unremarkable, and several stool specimens examined for parasites were negative; he had never had any eosinophilia. Based on respiratory symptoms, he underwent bronchoscopy, and *S. stercoralis* filariform larvae were recovered from sputum, bronchoalveolar lavage fluid, and three stool specimens. After treatment with thiabendazole, the patient remained on a prophylactic regimen of thiabendazole at 25 mg/kg twice a day for 2 days every month. In situations where information on the donor is not readily available (possible strongyloidiasis), the recipient should be monitored for evidence of clinical strongyloidiasis and prophylaxis may be justified, since most episodes of hyperinfection occur during the first 3 months following transplantation. Also, discontinuation of cyclosporin A treatment in a setting in which no prior screening for *Strongyloides* had taken place might place a patient at risk for dissemination (66). Specific conditions associated with immunosuppression and strongyloidiasis can be seen in Table 13.5 (63, 67, 68).

The possibility of hyperinfection also puts patients with asthma and intestinal strongyloidiasis at significant risk if their lung disease is treated with systemically administered corticosteroids. Apparently, fewer than 10 days of therapy with corticosteroids has led to disseminated strongyloidiasis (63). This potential situation demonstrates the need to screen all asthmatics who come from areas where *S. stercoralis* is endemic, including the southeastern United States, for the presence of this parasite. Various immunosuppressive drugs that have been associated with hyperinfection include the glucocorticoids (both high and low doses), vinca alkaloids (vincristine and vinblastine), and others, although in many cases glucocorticoids were given at the same time (63, 67, 69).

In addition to the actual tissue damage caused by the migrating larvae, the patient may die of sepsis and/or meningitis, primarily as a result of the intestinal flora being carried via the larvae into the blood and/or central nervous

TABLE 13.5 Strongyloidiasis: associated conditions related to immunosuppression[a]

Condition	Description	Comments
Immunosuppressive drugs		
Glucocorticoids	Both high- and low-dose steroids; can begin as early as 20 days after therapy started; suppression of eosinophilia and lymphocyte activation	Most directly linked with hyperinfection syndrome; used for lupus, lymphoma, rheumatoid arthritis, leprosy, polymyositis, corneal ulcer, and Bell's palsy
Vinca alkaloids	Vincristine; decreased intestinal motility, longer time for larvae to change from noninfectious to infectious	Several reports, but information is anecdotal
Cyclosporin A	No proven association; some evidence that drug may be anthelmintic	Widespread use, but does not appear to play a role in hyperinfection
Other drugs	Azathioprine, cyclophosphamide, antithymocyte globulin, anti-CD_w, chlorambucil, 6-mercaptopurine, methotrexate, bleomycin, adriamycin, doxorubicin, daunorubicin, ifosfamide, melphalan, carmustine, VP16, mitoxantrone, and total-body irradiation	Because glucocorticoids were administered at the same time, hyperinfection link to these drugs may or may not be correct.
Hematologic malignancies	Case reports of hyperinfection syndrome prior to therapy with immunosuppressives	Chronic parasite-induced inflammation may contribute to lymphoma.
Transplant recipients		
Kidney	Majority of transplant hyperinfection cases seen with kidneys	Probably caused by glucocorticoids in response to organ rejection
Heart	Case of graft rejection	Patient treated with increased doses of corticosteroids
Bone marrow	Few cases reported	Supposition that drugs (bisulfan, cyclophosphamide) may prevent egg hatching; therefore, hyperinfection would be rare in these cases
HTLV-1	Associated with increased strongyloidiasis, failure of conventional therapy, and hyperinfection syndrome	Suggested that serum IgE confers some protection against heavy infections and response may be inhibited by HTLV-1 infection
Hypogammaglobulinemia	Seen in case of multiple myeloma and nephrotic syndrome	Immunity to infective-stage larvae not protective against autoinfective larvae

[a] Adapted from references 63, 65, 67, and 69.

system. Cerebrospinal fluid cultures have grown *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enterococcus faecalis,* and *Streptococcus bovis,* as well as other isolates. Migrating larvae have been found in cerebrospinal fluid, meningeal vessels, the dura, and the epidural, subdural, and subarachnoid spaces (63). Other causes of death may include peritonitis, brain damage, or respiratory failure.

Few cases of reactive arthritis have been reported, and the number of cases may be underestimated. A case of reactive arthritis combined with uveitis associated with a long-standing, heavy *Strongyloides* infection was found in a 32-year-old patient positive for human T-cell leukemia virus type 1 (HTLV-1). Treatment with thiabendazole and ivermectin resulted in rapid improvement. A case of myelitis associated with eosinophilia in blood and cerebrospinal fluid was identified as being caused by *S. stercoralis*; apparently, this is the first report of myelitis in the course of strongyloidiasis (70).

HTLV-1 Infection. Coinfection with HTLV-1 and *S. stercoralis* is associated with dissemination of parasites and development of severe forms of strongyloidiasis. The prevalence of *S. stercoralis* is significantly higher in HTLV-1 carriers than HTLV-1 noncarriers in Japan and elsewhere, including Central Australia. Since cellular immunity plays a major role in the host defense against strongyloidiasis, infection with HTLV-1 appears to change the immune system capability, leading to severe clinical manifestations and disseminated disease (71). HTLV-1 infects predominantly T cells, leading to spontaneous proliferation of lymphocytes and increased secretion of cytokines. Since levels of IFN-γ and numbers of $CD4^+$ and $CD8^+$ T cells are decreased in HTLV-1 carriers coinfected with helminths, this indicates that helminthic infection may down-regulate production of IFN-γ in HTLV-1 carriers. It has also been documented that the prevalence of helminthic infection was 7-fold higher in HTLV-1 carriers than in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis. These data show that helminthic infection decreases activation of type 1 cells, which may influence the clinical outcome of HTLV-1 infection (72).

Hypereosinophilia, which is common in patients with mild strongyloidiasis, is often not seen in these patients and may be inhibited by the HTLV-1 infection. The relationship between *S. stercoralis,* infection with HTLV-1, and serum IgE levels reveals some very interesting information. The prevalence of *Strongyloides* infection is significantly higher in HTLV-1 carriers than in those without HTLV-1 infection. The level of IgE is low in HTLV-1 carriers and is significantly lower in HTLV-1 carriers than in noncarriers among people with *Strongyloides* infection. This information suggests that serum IgE may confer some degree of protection against heavy infections with *Strongyloides* and that this response may be inhibited by HTLV-1 infection.

HIV Infection. Although it is well known that many people are coinfected with HIV and *S. stercoralis,* few cases of hyperinfection have been found in this patient group, many of whom previously received steroids for *Pneumocystis* pneumonia or non-Hodgkin's lymphoma. Due to documented cases of posttreatment hyperinfection after therapy for intestinal infection, questions remain regarding screening of HIV-positive individuals for strongyloidiasis and treating infected patients with anthelmintics. However, empiric ivermectin therapy may be a reasonable approach in AIDS patients originating from *Strongyloides* endemic areas. With the lack of disseminated strongyloidiasis documented in the AIDS group, the Centers for Disease Control and Prevention (CDC) and WHO removed this condition from their list of signature opportunistic infections in 1987. Thus, current epidemiology suggests that HIV is not a risk factor for disseminated strongyloidiasis.

***S. fuelleborni* Infection.** Children suffering from the swollen belly syndrome caused by *S. fuelleborni* usually present with protein-losing enteropathy, abdominal distention, and respiratory distress at about 8 to 10 weeks of age. The diarrhea is generally not severe, and there is no significant fever. Severe hypoproteinemia without proteinuria is very characteristic and leads to peripheral edema and ascites. Egg counts are often greater than 100,000/g of stool. Dissemination has been reported and, untreated, the condition is associated with a high mortality (56). These infections are found in Africa and Papua New Guinea. *S. fuelleborni* occurs in African primates where infection can be shared with humans, while *S. fuelleborni kellyi* occurs exclusively in humans in New Guinea.

Diagnosis

Hematology. Eosinophilia may be the only indication of infection with *S. stercoralis*. However, although eosinophilia is a common finding in patients with chronic *Strongyloides* infection, it is unreliable as a predictor of hyperinfection. In chronic strongyloidiasis, eosinophilia may be present only intermittently, and the lack of eosinophilia while receiving immunosuppressant therapy in no way excludes the possibility of an underlying chronic *Strongyloides* infection.

Stool. Confirmation of the infection depends on recovery and identification of the adult worms, larvae, or eggs from the stool, duodenal material, or sputum. Fecal specimens may not be positive, even after the routine ova and parasite examination, including the concentration procedure, is performed. Numbers of larvae found in the stool also vary from day to day. It is well known that the detection of *S. stercoralis* larvae in the stools may be very difficult, particularly in patients with chronic, low-level infection (Table 13.6). This problem can be attributed to irregular and low output of the larvae, as well as the sensitivity of current diagnostic methods. Patterns of *S. stercoralis* detection vary widely among infected patients, and intermittent larval shedding can lead to inflated estimates of drug efficacy. It is important to remember that, prior to entering a patient in a clinical trial of drug efficacy, it has been recommended that four consecutive stool specimens should be examined for *S. stercoralis* to determine the patient's infection status.

Duodenal Contents. Some suggest that sampling of duodenal contents may recover larvae if stools are negative, while others think that duodenal sampling may not be effective. Special techniques that have been recommended for the recovery of duodenal contents include the Entero-Test capsule (see chapter 5).

Concentration Methods. There are also special concentration techniques (Baermann) and methods for larva culture (Harada-Mori, petri dish), which are discussed in detail in chapter 4. These techniques provide a means of recovery of larvae from large amounts of stool material, and often the filariform larvae are recovered. To differentiate the *Strongyloides* filariform larvae from those of the hookworm, one should examine the tail under the microscope. *Strongyloides* filariform larvae have a slit in the tail, while hookworm filariform larvae have a pointed tail (Fig. 13.37). Refer to Algorithm 13.5.

Agar Plate Cultures. Agar plate cultures are also recommended and tend to be more sensitive than some of the other available diagnostic methods (73); stool is placed onto agar plates, and the plates are sealed to prevent accidental infections and held for 2 days at room temperature. As the larvae crawl over the agar, they carry bacteria with them, thus creating visible tracks over the agar. The plates are examined under the microscope for confirmation of larvae, the surface of the agar is then washed with 10% formalin, and final confirmation of larval identification is made via wet examination of the sediment from the formalin washings (see chapter 4). The agar consists of 1.5% agar, 0.5% meat extract, 1.0% peptone, and 0.5% NaCl. This approach is thought to be more sensitive and has become more widely used (73, 74).

TABLE 13.6 Diagnostic methods for strongyloidiasis[a]

Method	Pros	Cons	Comments
Direct wet mount	Simple to perform	Sensitivity very low	Other methods much more sensitive
Concentration wet mount (formalin ethyl acetate concentration)	Simple to perform; centrifugation at 500 × *g* for 10 min	Sensitivity low for a single stool sample	Four stools recommended to determine patient's infection status
Permanent stained smear (trichrome, iron-hematoxylin)	Performed as a routine part of the ova and parasite examination	Larval morphology may be distorted	If anything seen, reexamine concentration sediments
Baermann concentrate	Examination of much larger amount of stool	Messy to set up, possible exposure to infectious larvae	Handle with gloves; decontaminate glassware, etc., when test completed
Harada-Mori, agar plate concentration	Considered "mini" Baermann procedures; relatively simple to set up	Will require checking for several days; not as sensitive as Baermann concentrate	Not routinely performed, but can be helpful
Agar plate culture	Often considered the most sensitive method for larval recovery and identification	Must be examined for 2 days before calling culture negative	More sensitive than formalin ethyl acetate concentration if larvae are present at <50/g of stool
Duodenal contents	Some studies indicate good sensitivity for larval recovery; use of the Entero-Test capsule helpful	Sensitivity debated; duodenal intubation invasive	Multiple stool exams may be more helpful, particularly if agar plate cultures set up
Serologic tests	More widely available; sensitive but not always specific; more accurate methods under development	May not distinguish present from past infection; sensitivity decreased in patients with HTLV-1 infections and hematologic malignancies	Sensitivity may be lower in travelers, but helpful in immigrants with chronic infection; gelatin particle agglutination reported more sensitive than ELISA
Coproantigen tests	Newer tests more sensitive and have greatly diminished cross-reactivity issues	Somewhat insensitive; more accurate methods under development	With test improvements, this test will provide an excellent method for screening patients; interpretation value unclear concerning chronic versus acute infections in patient.
Molecular tests	Tests have generally achieved 100% specificity; however, repeated stool testing may be required for necessary sensitivity; newer tests under development	Availability generally limited to research facilities	Amount of parasite-specific DNA present might be directly correlated with the intensity of the infection and the host immune response.
Skin test	Immediate hypersensitivity; potential use possible	Coinfection with HTLV-1 decreases sensitivity.	Further developmental work required, nothing currently available commercially

[a]Adapted from references 73, 74, and 76–80.

Serology. As they become more widely available, the application of enzyme-linked immunosorbent assay (ELISA), immunoblotting, and gelatin particle agglutination methods has been investigated for both the diagnosis and postchemotherapy evaluation of human strongyloidiasis (75–77). These methods appear to be both sensitive and specific and have demonstrated significant decreases in antibody levels after therapy. However, when treatment was not effective, the individuals did not show a significant fall in antibody titers. More detailed studies to determine appropriate intervals for serologic evaluation and criteria for successful cure are required before these tests become more widely used. Serology is a useful tool; however, it might overestimate the prevalence of disease due to cross-reactivity with other nematode infections. Only in areas where other parasitic diseases are not endemic can the possibility of cross-reactions be reasonably ruled out. Also, serology has difficulty distinguishing recent from past (and cured) infections. Even if there is a decline in antibody titers after treatment, the drop is slow and testing needs to be carried out at 6 to 12 months after treatment, which can cause a substantial loss to follow-up in a clinical trial. Although these serology tests are available in various reference laboratories, as well as the CDC, the results may not always be definitive. Of the serologic assays developed to date, the *S. stercoralis* ELISA based on crude larval antigen (CrAg-ELISA) has been shown to have the highest sensitivity (94.6%) and is currently in use at the CDC in the United States and elsewhere (78).

Newer techniques such as the luciferase immunoprecipitation system assay (LIPS), based on a 31-kDa recombinant antigen (termed NIE) from *S. stercoralis* and/or the recombinant antigen *S. stercoralis* immunoreactive antigen (SsIR), or the NIE-ELISA have shown promise in controlled settings (78).

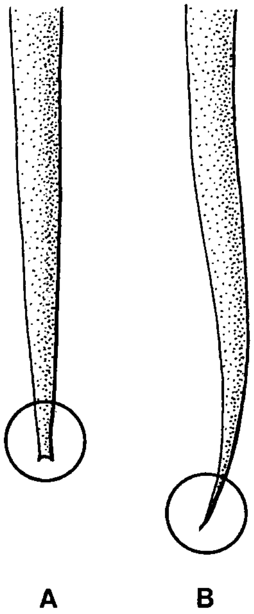
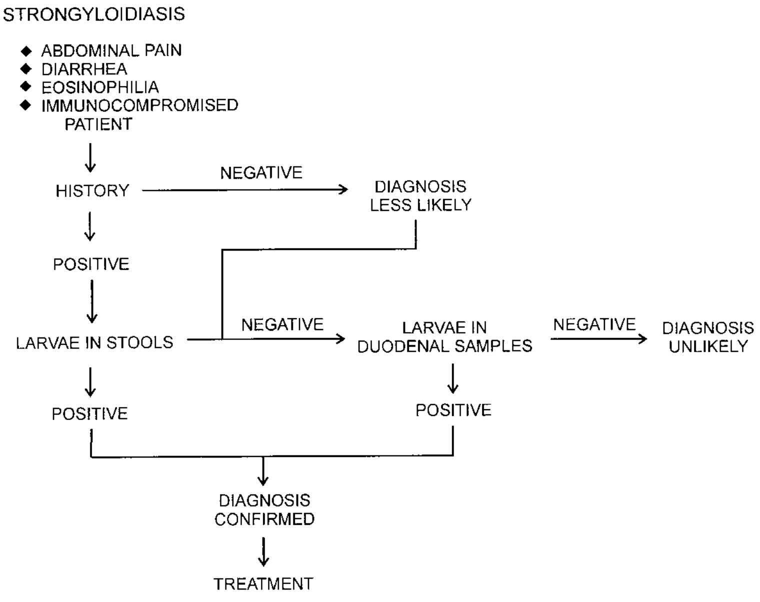


Figure 13.37 Tail (filariform larvae). (A) *Strongyloides stercoralis*; (B) hookworm. doi:10.1128/9781555819002.ch13.f37

Coproantigen Detection. Accurate diagnosis of infection with the parasite *S. stercoralis* is difficult due to the low number of larvae in stool, and most diagnostic methods are somewhat insensitive. An important issue in diagnostic assays for parasitic infections is the potential for cross-reactivity with antibodies or antigens from similar parasites, a discussion included in many nematode coproantigen and serodiagnostic method options including current *Strongyloides* antibody tests. However, a recent test shows a lot of promise and apparently can specifically detect *Strongyloides* antigens with no cross-reactivity with the other helminths tested (79).

Molecular Diagnostics. Several real-time PCRs have been designed for the detection of *S. stercoralis*, targeting either 18S rRNA, cytochrome *c* oxidase subunit I gene, or 28S RNA gene sequences in fecal samples. These tests have generally achieved 100% specificity (77). However, in asymptomatic patients with very low levels of larval output, the test is unlikely to achieve high sensitivity unless repeated stool samples are tested. Certainly the amount of fresh feces used for the molecular assays is 40 times lower than that required for the Baermann or agar plate culture method. Another consideration is the fact that the amount of parasite-specific DNA present might be directly correlated with the intensity of the infection and the host immune response. There remains an urgent need to develop new tests for the diagnosis of strongyloidiasis, and efforts are ongoing to improve the specificity of current and new serological methods and their value as a reliable cure marker.

Skin Tests. Due to the potential of internal autoinfection, even chronic asymptomatic infections with *S. stercoralis* have the potential to become severe disseminated disease with fatal outcome. Intermittent and scanty larval excretion makes parasitologic diagnosis difficult. Immunoreactivity of purified antigen with parasite-specific IgE from sera of *Strongyloides*-infected patients indicated its poten-


STRONGYLOIDIASIS
◆ ABDOMINAL PAIN
◆ DIARRHEA
◆ EOSINOPHILIA
◆ IMMUNOCOMPROMISED PATIENT
HISTORY
NEGATIVE
DIAGNOSIS LESS LIKELY
POSITIVE
LARVAE IN STOOLS
NEGATIVE
LARVAE IN DUODENAL SAMPLES
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
POSITIVE
DIAGNOSIS CONFIRMED
TREATMENT

Algorithm 13.5 Strongyloidiasis.
doi:10.1128/9781555819002.ch13.Alg13.5

tial use as an immediate sensitivity skin test antigen. This application of the antigen was supported by its capacity to trigger histamine release on *in vitro* exposure to blood from *Strongyloides*-infected patients and its failure to produce histamine release from blood of normal controls (80).

Immunocompromised Patients. Debilitated or immunocompromised patients should always be suspected of having strongyloidiasis, particularly if they experience unexplained bouts of diarrhea and abdominal pain, repeated episodes of sepsis or meningitis with intestinal bacteria, or unexplained eosinophilia. However, a recent comparative study of the occurrence of *Strongyloides* spp. in 554 AIDS and 142 non-AIDS patients demonstrated similar prevalences of infection in the two groups, thus indicating no significant statistical differences (81). Nevertheless, diagnosing intestinal parasites in HIV/AIDS patients is necessary, especially in those reported to be chronic alcoholics and those who are not receiving antiretroviral treatment. It is also important to check pre-hematopoietic stem cell transplantation patients in areas of endemic infection in the United States (69).

Diagnosis of *S. fuelleborni*. In contrast to *S. stercoralis*, the diagnosis of *S. fuelleborni* infection is made by finding eggs, rather than larvae, in the stool. Children with swollen belly syndrome tend to have very heavy infections and excrete large numbers of eggs daily. The eggs are oval and approximately 50 to 70 µm long (Fig. 13.35).

KEY POINTS—LABORATORY DIAGNOSIS

Strongyloides stercoralis

1. Recovery of the rhabditiform (noninfective) larvae is normally from the stool concentrate. *Caution: Filariform (infective)* larvae *can also be recovered in the stool.* A minimum of four stools are recommended before indicating that the patient is not infected (routine formalin, ethyl acetate sedimentation concentration); use centrifugation at 500 × *g* for 10 min. This remains a relatively insensitive method for diagnosis.
2. If the stool specimens are negative, examination of duodenal contents is recommended (duodenal aspirates, Entero-Test capsule); however, the overall sensitivity of the method varies.
3. Various concentrates (Baermann) and cultures (Harada-Mori, petri dish) can also be used for larval recovery. The benefit of examining a large sample of stool enhances these methods, particularly the Baermann concentration.
4. Eggs are rarely seen in the stool but may be recovered from duodenal contents.
5. In very heavy infections, eggs (less common), larvae (both types), and adult worms may be recovered in the stool. Also remember that larvae can be seen in respiratory specimens, particularly in hyperinfection cases.
6. If agar plates are used (culture, method of choice), they must be dried sufficiently to eliminate free water on the agar prior to use. If fresh agar plates have just been prepared, the drying time may be longer than if prepackaged commercial plates, which are shipped with little to no free moisture on the surface of the agar, are used. They need to be examined at 24 h and 2 days.

Treatment

At present, the recommended drug of choice is ivermectin, which cures 70 to 85% of chronic infections; however, thiabendazole has been used very successfully for many years. Specific dosages are provided in chapter 36. In some cases, repeated therapy is necessary, and the cure rates vary (63). Patients with the hyperinfection syndrome should be hospitalized during therapy for proper monitoring. Thiabendazole has been used to treat children with swollen belly syndrome due to *S. fuelleborni* infection. This approach seems to be effective, but reinfection is very likely to occur in areas of endemic infection.

Side effects with thiabendazole occur in most patients and include nausea, foul-smelling urine, neuropsychiatric effects, malaise, and dizziness. Rectal administration of ivermectin has also been reported and may be helpful in patients who cannot absorb or tolerate oral therapy (82).

Mebendazole has also been used to treat strongyloidiasis; however, it is poorly absorbed. Albendazole has also been used to treat patients with strongyloidiasis, as well as those with the hyperinfection syndrome. Few side effects have been seen, and this drug is an excellent alternative to ivermectin. It is important to remember that HTLV-1

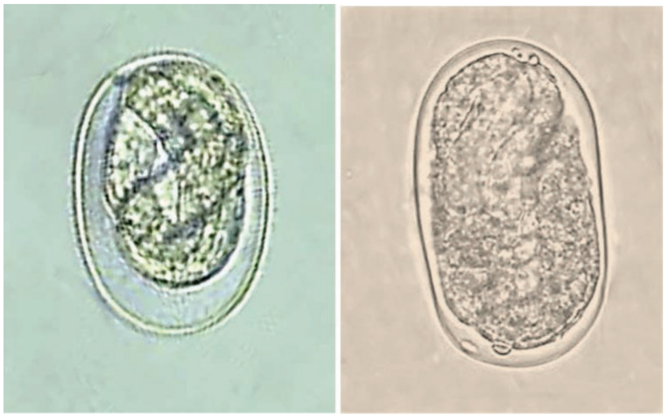


Figure 13.38 *Strongyloides fuelleborni* eggs. They measure within the same range as *S. stercoralis*; differentiation between the two species is very difficult. However, the patient's clinical history should be helpful.
doi:10.1128/9781555819002.ch13.f38

infection may be a possibility in patients with uncomplicated intestinal strongyloidiasis who do not respond to standard therapy (83). It is important to remember that, due to the risk of hyperinfection, all patients infected with *Strongyloides* should be treated.

Epidemiology and Prevention

Contact with contaminated infective soil, feces, or surface water should be avoided. Communities where close living conditions and poor sanitation facilities exist, including both rural and urban areas in the developing world, often have high prevalence rates of strongyloidiasis. However, *S. stercoralis* has the highest prevalence in the world in rural and remote Aboriginal communities of northern Australia. With prevalences greater than 25%, these communities have rates of strongyloidiasis even higher than those in the worst affected developing countries where surveys have been conducted (84). In some areas, some communities have estimates of up to 60% prevalence. Also, closed communities such as institutions for the mentally handicapped may have high rates of infection and transmission. The geographic distribution is quite broad and includes both tropical and temperate climates. It is important to remember that internal autoinfection allows for maintenance of the parasite within the host for years following the initial exposure. Since all infected individuals are at risk for hyperinfection and disseminated disease, the identification of people who may have contracted their infection many years earlier is of great clinical significance. Individuals found to have the infection should be treated. *All patients who are going to receive immunosuppressive drugs should be screened for strongyloidiasis before therapy.*

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14

Tissue Nematodes

- *Trichinella* spp.
- *Baylisascaris procyonis*
- *Lagochilascaris minor*
- *Toxocara canis* and *T. cati* (visceral larva migrans and ocular larva migrans)
- *Ancylostoma braziliense* and *A. caninum* (cutaneous larva migrans)
- Human eosinophilic enteritis
- *Dracunculus medinensis*
- *Angiostrongylus* (*Parastrongylus*) *cantonensis* (cerebral angiostrongyliasis)
- *Angiostrongylus* (*Parastrongylus*) *costaricensis* (abdominal angiostrongyliasis)
- *Gnathostoma spinigerum*
- *Gnathostoma doloresi*, *G. nipponicum*, *G. hispidum*, and *G. binucleatum*
- *Anisakis simplex*, *A. physetesis*, *Pseudoterranova decipiens*, *Contracaecum osculatum*, *Hysterothylacium aduncum*, and *Porrocaecum reticulatum* (larval nematodes acquired from saltwater fish)
- *Capillaria hepatica*
- *Thelazia* spp.

Trichinella spp.

Although *Trichinella spiralis* was first seen in human tissue at autopsy in the early 1800s, it was not until 1860 that Freidrich von Zenker concluded that the infection resulted from eating raw sausage (1). The consumption of rare or raw pork as the cause of trichinosis (also known as trichinellosis) was experimentally proved a few years later. By the 1900s, trichinosis was definitely recognized as a public health problem. This particular infection has a cosmopolitan distribution but is more important in the United States and Europe than in the tropics or Asia. The prevalence in autopsies within the United States has declined from 15.9% of human diaphragms studied at autopsy from 1931 to 1944 to 4.5% from 1948 to 1963; in recent years, the prevalence has decreased to 2.2% and the mortality associated with this infection has decreased to less than 1%. In 1990, only 105 cases of human trichinosis were reported in the United States, and by 1994 the number had dropped to 35 (2). During 1997 to 2001 the incidence decreased to a median of 12 cases annually and no reported deaths (3). However, in many areas of the world, trichinosis remains a problem (4).

During the 5-year period from 1997 to 2001, 72 cases were reported to the U.S. Centers for Disease Control and Prevention (CDC). Of these, 31 cases were associated with eating wild game: 29 with bear meat, 1 with cougar meat, and 1 with wild-boar meat (3). In comparison, only 12 cases were associated with eating commercial pork products; 4 of these cases were traced to a foreign source. Nine cases were associated with eating noncommercial pork from home-raised or direct-from-farm swine where U.S. commercial pork production industry standards and regulations are not applicable.

Studies of isolates of *Trichinella* spp. from Arctic, temperate, and tropical areas have confirmed that there are major differences related to their genetic structure and overall biology. Various species are involved, depending on the geographic area. *Trichinella* forms a complex of species, all of which appear to be the same morphologically but, based on DNA studies and comparative features, are actually quite different (Tables 14.1 and 14.2) (5). There are now nine recognized *Trichinella* species and three additional genotypes, *T. spiralis*,

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TABLE 14.1 *Trichinella* taxonomy and distribution

Species	Genotype	Host	Distribution
T. spiralis	T1	Mammals	Cosmopolitan
T. nativa	T2	Mammals	Arctic and subarctic regions of America, Europe, and Asia
	T6	Mammals	Arctic and subarctic regions of America
T. britovi	T3	Mammals	Temperate areas of Europe and Asia, Northern and Western Africa
	T8	Mammals	South Africa and Namibia
T. pseudospiralis	T4	Mammals and birds	Cosmopolitan
T. murrelli	T5	Mammals	Temperate areas of North America
	T9	Mammals	Japan
T. nelsoni	T7	Mammals	Eastern and Southern Africa
T. papuae	T10	Mammals and reptiles	Papua New Guinea
T. zimbabwensis	T11	Mammals and reptiles	Africa south of the Sahara
T. patagoniensis	T12	Mammals	Neotropics, Argentina

T. nativa, *T. nelsoni*, *T. britovi*, *T. pseudospiralis*, *T. murrelli*, T6, T8, T9, *T. papuae*, *T. zimbabwensis*, and *T. patagoniensis* (6–11). *Trichinella* is quite different from many other helminths because all stages of development (adult and larva) occur within a single host. More than 100 different mammals are susceptible to infection, and the cysts can remain viable and infectious for many years, even in decaying muscle tissue. These factors ensure successful transmission and the survival of the parasite.

Life Cycle and Morphology

Human infection is initiated by the ingestion of raw or poorly cooked pork, bear, walrus, or horse meat or meat from other mammals (carnivores and omnivores) containing viable, infective larvae (Fig. 14.1; Table 14.3). The minimum infectious dose for humans has not been clarified; however, it has been estimated that approximately 100 to 300 larvae cause disease. The tissue is digested in the stomach, and the first-stage larvae (L1) are resistant to gastric juice. The excysted larvae then invade the intestinal mucosa, develop through four larval stages within about 48 h, mature, and mate by the second day. By the fifth day of infection, the female worms begin to deposit motile larvae, which are carried by the blood vessels, intestinal lymphatic system, or mesenteric venules to the body tissues, primarily highly oxygenated striated muscle (Fig. 14.2 and 14.3). Deposition of larvae continues for approximately 4 to 16 weeks, with each female producing up to 1,500 larvae in the nonimmune host. Newborn larvae can penetrate almost any tissue but can continue their development only in striated muscle cells. With the exception of *T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis*, invasion of striated muscle cells stimulates the development of nurse cells (Table 14.1) (4, 12). As the larvae begin to coil, the nurse cell completes the formation of the cyst within about 2 to 3 weeks. The nurse cells presumably function to nourish the parasite as well as to protect it from the host immune response. Within the human host, the cyst measures about 400 by 260 µm, and within the cyst, the coiled larva measures 800 to 1,000 µm in length. At this point, the larvae are fully infective. After weeks to years, the calcification process occurs. It has been estimated that, in muscle nurse cells, parasite larvae can survive for up to 40 years in humans and over 20 years in polar bears.

Maintenance of a long-term host-parasite relationship requires the parasite to remain metabolically active by nutrient acquisition and waste disposal. The parasite attracts a highly permeable set of blood vessels to the surface of the outer capsule, thus providing a constant source of small-molecular-weight metabolites while also removing metabolic waste products (12). In nonencapsulated *Trichinella* infections, the vessels surrounding nurse cells apparently exist prior to parasite invasion of muscle cells.

The very active muscles including the diaphragm; the muscles of the larynx, tongue, jaws, neck, and ribs; the biceps; and the gastrocnemius, which have the greatest blood supply, are invaded. The encysted larvae may remain viable for many years, although calcification can occur within less than a year. As few as five larvae/g of body muscle can cause death, although 1,000 larvae/g have been recovered from individuals who died from causes other than trichinosis.

There are species differences in low-temperature (freezing) survival, infectivity, and capsule formation (4). Also, studies on a pig farm indicate that, even in the absence of a known source of infected meat (garbage containing meat scraps or dead animals), the rat population maintained the infection, probably through cannibalism. Consequently, to reduce transmission of *T. spiralis* between rats and swine, rat populations in an agricultural ecosystem must be controlled. It is also important to limit access to the farmyard by wild and feral animals.

Although recommendations have been made to use several species designations, some publications still use the

TABLE 14.2 *Trichinella* species and genotypes[a]

Species or genotype	Geographical distribution	Host range	Pathogenicity for humans; main source of human infections	Resistance of larvae in frozen muscles
Encapsulated				
T. spiralis	Worldwide, most important species for human infections	Domestic and sylvatic mammals	High; pigs, rats, horses	Yes in horse muscles
T. nativa	Arctic and subarctic areas of America, Asia, Europe	Sylvatic carnivores, bears, walrus, whales, seals, squirrels, dogs, wolves, foxes, horses	High; bears, walruses	Yes in carnivore muscles
Trichinella genotype T6	Canada, Alaska, Rocky Mountains, and Appalachian Mountains in the United States, possibly as far as Sierra Madre Mountains in Mexico	Sylvatic carnivores, including grizzly bears	Carnivores, bears	Yes in carnivore muscles
T. britovi	Temperate areas of Europe and Asia, Northern and Western Africa; second-most common species impacting human health	Sylvatic mammals and less common for domestic pigs, foxes, wolves, wild bears, horses, free-ranging swine	Moderate; bush pigs, warthogs, wild boars, domestic pigs, horses, foxes, jackals	Yes in carnivore and horse muscles
Trichinella genotype T8	South Africa and Namibia	Sylvatic carnivores	None documented	No
T. murrelli	United States and southern Canada	Sylvatic carnivores, no development in swine	Bears, horses	No
Trichinella genotype T9	Japan	Sylvatic carnivores, foxes, raccoon dogs, brown bears, raccoons	None documented	No
T. nelsoni	Eastern-southern Africa	Sylvatic mammals, bush pig, warthog, lions, leopards, cheetahs, hyenas	Low; warthogs, bush pigs	No
T. patagoniensis	Neotropics, Argentina	Cougars	None documented	Yes at −5°C; no at −18°C
Nonencapsulated				
T. pseudospiralis	Worldwide	Sylvatic mammals and birds, domestic pigs, raccoons, rodents	Low to moderate; domestic and wild pigs	No
T. papuae	Papua New Guinea, Thailand	Wild pigs, saltwater crocodiles	Wild pigs	No
T. zimbabwensis	Zimbabwe, Mozambique, Ethiopia, South Africa	Nile crocodiles, monitor lizards, farmed reptiles	None documented, but suspected	No

[a] Adapted from references 10 and 19.

single species designation *Trichinella spiralis* (Tables 14.2 and 14.3). Genetic relationships among many *Trichinella* isolates are currently being assessed by dot blot hybridization, restriction endonuclease, and gel electrophoresis techniques. On the basis of the presence of repetitive DNA sequences in the *Trichinella* genome, distinctive banding patterns have been seen among the isolates, and taxonomic changes will continue to occur (13).

Clinical Disease

Pathologic changes due to trichinosis can be classified as (i) intestinal effects and (ii) muscle penetration and larva encapsulation (Table 14.4). Any damage caused in either phase of the infection is usually based on the original number of ingested cysts; however, other factors such as the patient's general health, age, and size also play a role in the disease outcome. Symptoms of trichinosis are generally separated into three phases, with phase 1 being related to the presence of the parasite in the host prior to muscle invasion and phase 2 being related to the inflammatory and allergic reactions due to muscle invasion. There may also be an incubation period of up to 50 days. Phase 3 is the convalescent phase or chronic period (Table 14.5).

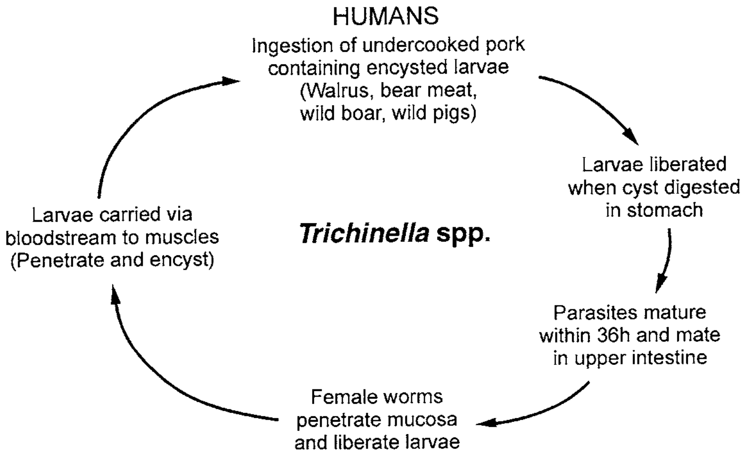

HUMANS
Ingestion of undercooked pork containing encysted larvae (Walrus, bear meat, wild boar, wild pigs)
Larvae liberated when cyst digested in stomach
Parasites mature within 36h and mate in upper intestine
Female worms penetrate mucosa and liberate larvae
Larvae carried via bloodstream to muscles (Penetrate and encyst)
Trichinella spp.

Figure 14.1 Life cycle of *Trichinella* spp. A number of infected meat sources other than pork are relevant for the various species of *Trichinella*.
doi:10.1128/9781555819002.ch14.f1

Symptoms that may develop within the first 24 h include diarrhea, nausea, abdominal cramps, and general malaise, all of which may suggest food poisoning, particularly if several people are involved. Studies also indicate that the diarrhea can be prolonged, lasting up to 14 weeks (average, 5.8 weeks) with few or no muscle symptoms. It is still unknown whether this clinical presentation is related to variant biological behavior of Arctic *Trichinella* organisms, to previous exposure to the parasite, or to other factors.

During muscle invasion, there may be fever, facial (particularly periorbital) edema, and muscle pain, swelling, and weakness. Other signs are conjunctivitis, headache, dry cough, petechial bleedings, and painful movement disorder of the eye muscles (Fig. 14.4). The extraocular muscles are usually the first to be involved, followed by the muscles of the jaw and neck, limb flexors, and back. Muscle damage may cause problems in chewing, swallowing, breathing, etc., depending on which muscles are involved. The most severe symptom is myocarditis, which occurs in approximately 5 to 20% of cases and which usually develops after the third week. Symptoms include pericardial pain, tachycardia, and electrocardiogram abnormalities such as nonspecific ventricular repolarization disturbances, followed by bundle-branch conduction disturbances, and sinus tachycardia (14). Reversal of the potassium deficit corrects the electrocardiogram abnormalities. Screening should be performed for all patients suspected of having trichinosis; serum troponin provides a simple and reliable means and can be positive even in asymptomatic myocarditis.

Death may occur between the fourth and eighth weeks. Other severe symptoms, which can occur at the same time, may involve the central nervous system (CNS). Although *Trichinella* encephalitis is rare, it is life-threatening. Technological advances such as the computed tomogram, angiogram, and electroencephalogram are of no diagnostic assistance and probably add nothing to traditional diagnostic information, which includes eosinophilia, sedimentation rate, and muscle biopsy.

It is estimated that 10 to 20% of the patients with trichinosis have CNS involvement and that the mortality rate may reach 50% in these patients if they are not treated. Symptoms may mimic those of polyneuritis, acute anterior poliomyelitis, myasthenia gravis, meningitis, encephalitis, dermatomyositis, and polyarteritis nodosa. There may be focal paresis or paralysis (quadriplegia to single muscle group).

Peripheral eosinophilia of at least 20%, often over 50% and possibly up to 90%, is present during the muscle invasion phase of the infection. Fever can also be present at this time and can persist for several days to weeks, depending on the intensity of the infection. However, once the larvae begin to encapsulate, patient symptoms subside; eventually the cyst wall and larvae calcify.

In an outbreak in Spain, 44 members of eight families were examined. Various people had suggestive symptoms (10 of 44), hypereosinophilia (20 of 44), and positive serologic test results (15 of 44). Three groups could be identified according to the home-prepared product each had ingested (pork sausage, blood pudding, and loin). In these cases, the common source of all infections was the poorly cooked pork sausage, since the blood pudding is boiled for a long time at high temperature and the loin is always served thoroughly fried or roasted. Twelve months later, all had a normal eosinophil count and negative serologic test results (15). Another outbreak in Spain involved 38 people, 15 of whom were hospitalized after the ingestion of sausage made from uninspected wild boar meat and infected pork. Almost all patients had myalgias, about half reported diarrhea and/or vomiting, 75% reported periorbital edema, and 76% had fever. Sixteen patients were positive for *T. britovi* by indirect fluorescent-antibody test (IFAT) and 20 were positive by Western blotting (16). The ingestion of wild boar containing *T. pseudospiralis* in

TABLE 14.3 Tissue nematodes

Name	How acquired	Location in body	Symptoms	Diagnosis
Trichinella spiralis (*T. nativa*) (*T. nelsoni*) (*T. britovi*) (*T. pseudospiralis*) (*T. murrelli*) (*Trichinella* T6) (*Trichinella* T8) (*Trichinella* T9) (*T. papuae*) (*T. zimbabwensis*) (*T. patagoniensis*)	Ingestion of raw or rare meats (pork, bear, walrus, other carnivores and/or omnivores)	Active muscles contain encysted larvae (diaphragm, tongue, larynx, neck, ribs, biceps, gastrocnemius)	Diarrhea (larval migration through intestinal mucosa), nausea, abdominal cramps, general malaise; muscle invasion: periorbital edema, pain, swelling, weakness, difficulties in swallowing, breathing, etc.; most severe symptom is myocarditis; high eosinophilia (20–90%)	Biopsy or autopsy specimen (muscle) compression smear or routine histology; artificial digestion of muscle to release larvae (larvae are very infective and precautions should be taken); serologic testing can be very helpful
Baylisascaris procyonis	Ingestion of viable eggs in the soil (most probably from raccoon feces)	CNS and eye contain larvae	Eosinophilic meningitis, unilateral neuroretinitis	Biopsy or autopsy specimen, routine histology; eggs from raccoon feces measure 80 μm long by 65 μm wide, have a thick shell with a finely granulated surface, and resemble *Ascaris lumbricoides* eggs
Lagochilascaris minor	Life cycle and route of human infection unknown; suspect ingestion of viable eggs in the soil	Adult worms, larvae, and eggs occur in life cycle within human lesions (neck, throat, nasal sinuses, tonsillar tissue, mastoids, brain, lungs)	Pustule swelling, pus in lesions; chronic granulomatous inflammation	Identification of adult worms, larvae, or eggs from lesions, sinus tracts, or biopsy or autopsy specimens
Toxocara canis and *T. cati* (visceral and ocular larva migrans)	Ingestion of infective eggs (dog/cat ascarids) from fecal material in the soil	Usually the liver; migratory pathway may include the lungs and even back to the intestine	Migration of larvae may cause inflammation and granuloma formation; there may be fever, hepatomegaly, pulmonary infiltrates, cough, and neurologic symptoms; high eosinophilia (up to 90%; 20–50% common)	Confirmation at autopsy; serologic test (ocular fluids as well as serum if eye involved)
Ancylostoma braziliense and A. caninum (cutaneous larva migrans)	Skin penetration of filariform/infective larvae of dog/cat hookworms; infection can also occur via ingestion of infective larvae	Larval migration in the skin produces linear/raised/vesicular tracts; can be on any area of the body	Intense itching, pneumonitis (if larvae migrate to deeper tissues)	Picture of linear tracts; possible removal of larva from tunnel
Dracunculus medinensis (fiery serpent)	Ingestion of infected copepod/water flea (*Cyclops*)	Adult worms develop in deep connective tissue; gravid female migrates to feet and ankles (can occur anywhere), where blister forms for larval deposition into the water through the ruptured blister on the skin	Before blister formation: erythema, tenderness, urticarial rash, intense itching, nausea, vomiting, diarrhea, or asthmatic attacks; if secondary infection occurs, there may be cellulitis, arthritis, myositis, etc.	Formation of cutaneous lesion with appearance of adult female worm depositing larvae into the water; calcified worms can also be found on X ray
Angiostrongylus cantonensis (eosinophilic meningitis) (cerebral)	Accidental ingestion of infective larvae in slugs, snails, or land planarians	Brain tissue, eye (rare), lung tissue (rare)	Severe headache, convulsions, limb weakness, paresthesia, vomiting, fever, eosinophilia up to 90%	Presumptive: severe headache, meningitis or meningoencephalitis, fever, ocular involvement; definitive: examination of tissues (surgical specimens)
Angiostrongylus costaricensis (abdominal)	Accidental ingestion of slugs, often on contaminated salad vegetables	Bowel wall	Pain, tenderness, palpable tumor-like mass in right lower quadrant, fever, diarrhea, vomiting, eosinophilia (60%), and leukocytosis	Worm recovery and clinical history

(continued)

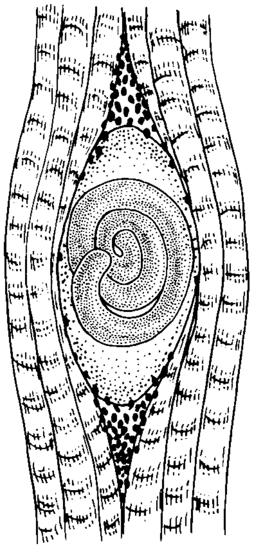
TABLE 14.3 Tissue nematodes *(continued)*

Name	How acquired	Location in body	Symptoms	Diagnosis
Gnathostoma spinigerum	Ingestion of raw, poorly cooked, or pickled freshwater fish, chicken (and other birds), frogs, or snakes	Migration of larvae in deep cutaneous or subcutaneous tissues (may appear anywhere), eyes, or CSF (less common)	Migratory swellings (hard, nonpitting) with inflammation, redness, pain	Worm recovery and clinical history
Anisakis, *Contracaecum*, *Pseudoterranova*, *Hysterothylacium*, and *Porrocaecum* spp.	Ingestion of raw, pickled, salted, or smoked saltwater fish	Wall of gastrointestinal tract	Nausea, vomiting; may mimic gastric/duodenal ulcer, carcinoma, appendicitis; stool positive for occult blood	Worm recovery and clinical history
Capillaria hepatica	Accidental ingestion of eggs from soil	Liver	May mimic hepatitis, amebic abscess, or other infections involving the liver	Histologic identification
Thelazia spp.	Larval deposition by flies	Conjunctival sacs/migrating over cornea	Excessive lacrimation, itching, pain (feeling of foreign object in eye)	Worm recovery (from eye) and identification

France and bear meat containing *T. nativa* from New York and Tennessee has also been implicated in recent outbreaks (17, 18).

There have also been a number of outbreaks due to consumption of horse meat; these outbreaks were caused by different species of *Trichinella* and were associated with differences in clinical symptoms. Although most human infections have been attributed to *T. spiralis*, these outbreaks clearly demonstrate that different species produce different clinical syndromes (Table 14.6) (19).

Figure 14.2 *Trichinella* spp., encysted larva in muscle. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch14.f2



Diagnosis

The European Centre for Disease Control has issued a case definition to be used when a human trichinosis case or outbreak is suspected (Table 14.7) (14). An algorithm for the diagnosis of acute trichinosis and for defining very unlikely, suspected, probable, highly probable, and confirmed cases is shown in Table 14.8 (14).

Depending on the severity of the infection, trichinosis can mimic many other conditions. Most mild cases with a small loading dose of infective larvae may present with flu-like symptoms. Unless the clinician recognizes an appropriate history, fever, myalgia, periorbital edema, and/or rising eosinophilia (50% or higher), the cause may go undetected (Table 14.9) (19, 20). Often, the first clue is the patient's history of possible ingestion of raw or rare pork or other infected meat. There may also be other individuals from the same group with similar symptoms. Trichinosis should always be included in the differential diagnosis of any patient with periorbital edema, fever, myositis, and eosinophilia, regardless of whether a complete history of consumption of raw or poorly cooked pork is available. If present, subconjunctival and subungual splinter hemorrhages also add support for such a presumptive diagnosis. If the meat consumption history is incomplete, food poisoning, intestinal flu, or typhoid may be suspected. It is very rare to recover adult worms or larvae from stool or other body fluids (blood, cerebrospinal fluid [CSF], etc.), even if the patient has diarrhea.

Muscle Biopsy. Muscle biopsy (gastrocnemius, deltoid, and biceps) specimens may be examined by compressing the tissue between two slides and checking the preparation

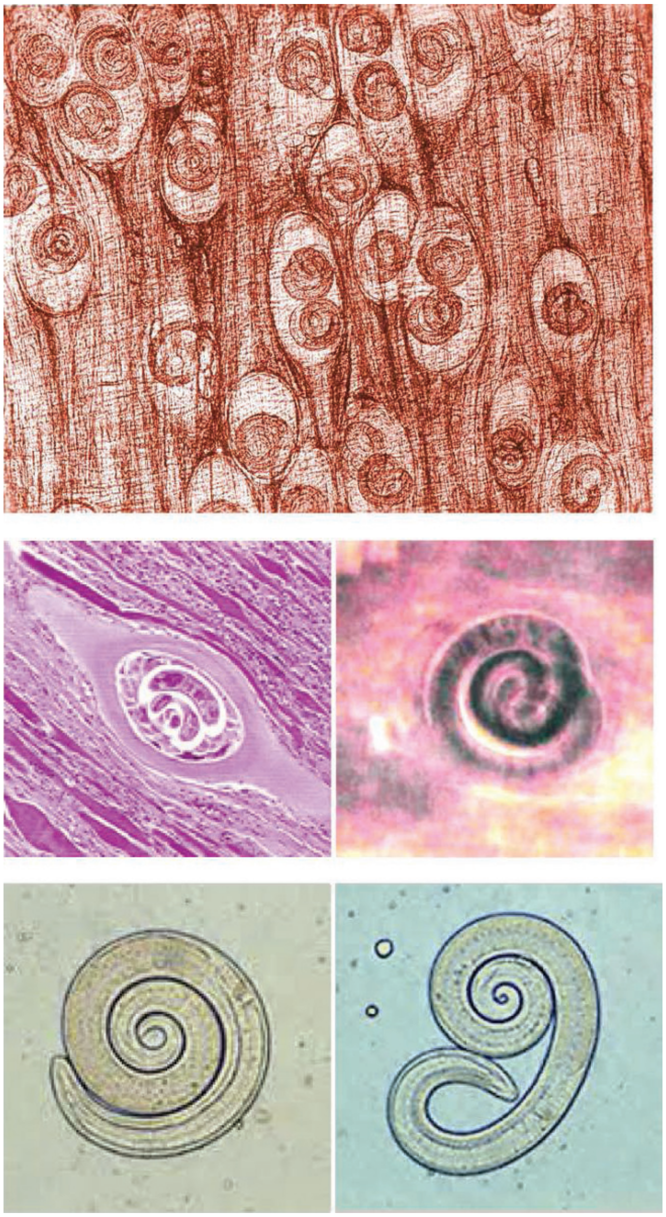


Figure 14.3 (Upper) *Trichinella spiralis*, encysted larva in muscle. (Middle, left) *Trichinella spiralis*, encysted larva in muscle; adjacent muscle has been replaced by fibrous tissue and is infiltrated with chronic inflammatory cells (×85) (Armed Forces Institute of Pathology photograph). (Middle, right) Coiled larva of *T. spiralis* in a teased muscle preparation (wet mount, ×193) (Armed Forces Institute of Pathology photograph). (Lower) *Trichinella* larvae, wet mounts from tissue digestion (may be recovered in stool within first 24 h of infected meat ingestion).
doi:10.1128/9781555819002.ch14.f3

under a microscope at low power (10× objective). However, this method does not provide positive results until 2 to 3 weeks after the onset of the illness. It is also important to remember that not all species form the capsule (Fig. 14.5). Muscle specimens or samples of the suspect meat can also be examined by using an artificial digestion technique to release the larvae. These techniques are described in detail in chapter 6.

Antibody Detection. Serologic tests are also very helpful, the standard two being the enzyme immunoassay (EIA) and the bentonite flocculation test, which are recommended for trichinosis. The EIA is used for routine screening, and all EIA-positive specimens are tested by bentonite flocculation for confirmation. A positive reaction in both tests indicates infection with *T. spiralis* within the previous few years. Often, antibody levels are not detectable within the first month postinfection. The titers tend to peak in the second or third months postinfection and then decline over a period

TABLE 14.4 *Trichinella spiralis*: life cycle stages and clinical conditions[a]

Stage in life cycle	Time after infection when symptoms begin	Clinical condition
Excysted larvae enter intestinal mucosa	2–4 h–24 h	Gastrointestinal symptoms
Worms mature and mate	30 h	
Females deposit larvae; muscle invasion begins via migration in lymphatic and blood vessels to highly oxygenated muscles	Day 6–day 7	Facial edema/fever
Heaviest muscle invasion	Day 10–day 11	Maximum fever (40–41°C); muscle inflammation/pain
Decrease in larval deposition	Day 14	Eosinophilia/antibody
Larvae differentiated	Day 17–day 20	Maximum eosinophilia
Encapsulation of larvae	Day 21	Myocarditis/neurologic symptoms
Intestine free of adult worms	Day 23–day 26	Respiratory symptoms
Encapsulation almost complete	Mo 1–mo 2	Fever subsides
Adult worms die	Mo 3	Death from myocarditis or encephalitis
Cyst calcification begins	Mo 6–mo 8	Slow convalescence; myocarditis/neurologic symptoms subside
Cyst calcification usually complete	Yr 1	
Most larvae still viable within calcified cyst	Yr 6	

[a] Adapted from reference 1.

TABLE 14.5 Trichinosis: incubation period, larval numbers, and degree of illness

Characteristic	Mild disease	Moderate disease	Severe disease	Abortive disease
No. of larvae/g of muscle	10, probably subclinical	50–500	1,000 or more	Less than 10
Incubation period (days)	21	16	7	30
Intestinal phase (phase 1) (days 2–7)[a]	Nausea, abdominal aches, cramps, loss of appetite, vomiting, mild fever, mild diarrhea or constipation; frontal headaches, dizziness, weakness	As with mild disease, symptoms may mimic the flu	Same symptoms as indicated in mild to moderate disease; diarrhea may be severe	Patient may be asymptomatic
Muscle invasion phase (phase 2) (days 9–28)[b]	Penetration of larvae initiates inflammatory response (extraocular muscles) in masseters; muscles of the larynx, tongue, diaphragm, and neck; intercostals; and muscular attachments to tendons and joints. Headache, fainting, urticaria, splinter hemorrhages beneath the fingernails and toenails, conjunctivitis, loss of appetite, hoarseness, dysphagia, dyspnea, and edema of the legs may also occur; range of symptoms is based on number of larvae and general health, age, size of patient		Muscular pain, facial edema (swelling of eyelids), fever, chills, eosinophilia, tachycardia, coma, respiratory difficulties; neurologic symptoms may be severe (may stimulate meningitis); myocarditis is a serious complication, may lead to congestive heart failure	Symptoms may or may not be seen
Convalescent phase (phase 3)	Decrease in muscular symptoms, beginning in the second month; fever and itching subside		Evidence of congestive heart failure may appear (if patient becomes active too soon)	Change in symptoms may or may not be obvious

[a] Symptoms reflect mucosal irritation.

[b] Encystment occurs after day 14.

of a few years. The IFAT has also been used to track the course of disease after infection. Antibody detection using IFAT was reported in 70.2% of patients 1 week after onset of disease and in 91, 94.3, and 100% at 2, 3, and 4 weeks, respectively, after the onset. Four months after therapy the antibody detection decreased to 25% (21). More information on serologic testing is presented in chapter 33. Refer to Algorithm 14.1 for a detailed review.

A dot enzyme-linked immunosorbent assay (ELISA) with purified antigens has been developed for detecting *T. spiralis* in swine. This test is as sensitive as an ELISA with excretory-secretory products as the antigen and Western immunoblot analysis and is nearly as specific as the Western blot. Also, the dot ELISA is much easier to perform than is a Western blot analysis (22).

Antigen Detection. A newly developed *T. spiralis* cathepsin B-like protease gene circulating antigen has been developed. Using an improved double-antibody sandwich ELISA, the antigen can be detected much earlier than antibody detection. Also, in this mouse model, the levels of circulating antigen dramatically decreased after successful therapy, while the antibody level remained unchanged. Hopefully, this approach could be adapted for use in humans as well (23).

A modified double-sandwich ELISA has been developed using polyclonal antibodies against larval somatic antigens. In experimental studies, the first detection of coproantigen occurred as early as day 1 postinfection, peaking on day 7, and then disappearing by week 3. These results were confirmed using the coagglutination test. Based on these studies, this approach could be used to confirm early infection in humans (24).

KEY POINTS—LABORATORY DIAGNOSIS

***Trichinella* spp.**

1. The history and clinical findings may suggest possible trichinosis (consumption of rare or raw infected meat). Remember to check hematology results for a possible eosinophilia (can reach 50% or higher).
2. Using compression slides, examination of suspect meat may reveal larvae (artificial digestion procedure) (see chapter 6). Note that not all species of larvae form the capsule; however, the unencapsulated larvae can still be seen in a "squash" preparation of biopsy material.
3. Larvae or adult worms are rarely recovered in fecal specimens during the intestinal phase (diarrhea).
4. Examination of muscle tissue obtained at biopsy may confirm the diagnosis (tissue compression between two slides, routine histology, or the artificial digestion technique).

(continued)

KEY POINTS—LABORATORY DIAGNOSIS

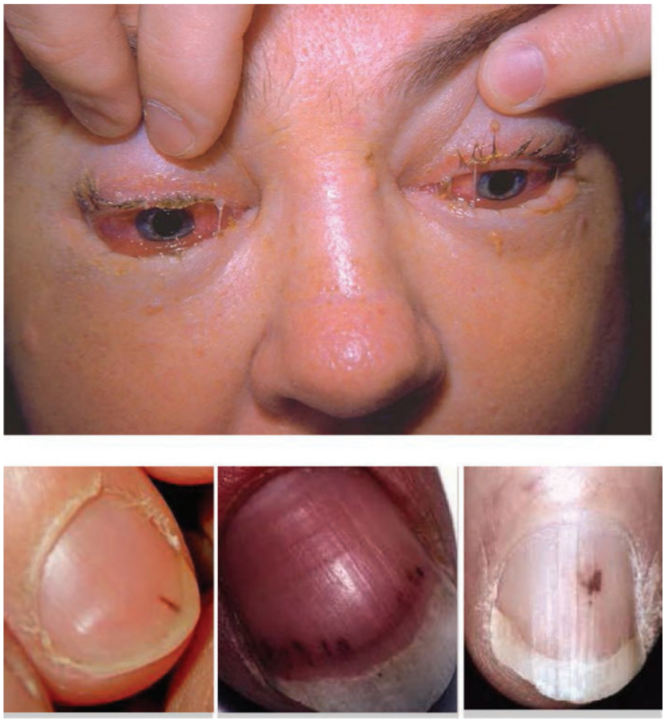
Trichinella spp. *(continued)*

5. Serologic tests for antibody detection may be very helpful; coproantigen detection tests are being developed.
6. Troponin results can confirm myocarditis, even in asymptomatic patients.
7. Eosinophilia is usually pronounced, but may be absent (25).

Treatment

Therapy depends on the phase of the disease, the immune status of the patient, and the intensity and length of the disease. For the early phase of infection, the objective is to reduce the number of larvae that will invade the muscles. Once larval invasion of the muscles has occurred, the objective becomes to reduce muscle damage. The current recommendation for the gastrointestinal phase is the use of mebendazole (200 to 400 mg/day for 3 to 5 days) or albendazole (400 mg/day); after the first 3 to 5 days of mebendazole therapy, the dose is changed (400 to 500 mg/day) and given for 10 days. In some cases, mebendazole and albendazole are given for persistent muscle pain (26). For acute, severe infection, steroids are recommended (prednisolone, 40 to 60 mg/day) along with mebendazole (5 mg/kg/day) or albendazole (400 to 800 mg twice a day for 8 to 14 days). For moderate or mild infection, steroids can be given as required; once fever and allergic signs diminish, steroid administration can be discontinued. Unfortunately, the disease is often not diagnosed until well after muscle invasion has begun. At this point, supportive therapy may be the only option.

Figure 14.4 (Upper) Trichinosis, periorbital swelling, eye irritation (public domain, courtesy of Thomas F. Sellers, Emory University and the CDC Public Health Image Library). (Lower) Examples of fingernail splinter hemorrhages in trichinosis (courtesy of CDC Public Health Image Library). doi:10.1128/9781555819002.ch14.f4



Epidemiology and Prevention

The *Trichinella* cycle that is maintained in nature occurs among cannibalistic and carrion-feeding carnivores. While domestic pigs and rats tend to be secondary hosts, the majority of human infections have been from infected pork. In reviewing data on trichinosis in the United States from 1997 to 2001, pork was implicated in 30% of the cases and wild game was implicated in 43% (3). Sausage was the most frequently implicated pork product and was often from noncommercial sources (3). While cases acquired from pork consumption continue to decline, the proportion of cases acquired from wild game meat has increased. However, the absolute numbers remain at about 9 to 12 per year. Continued multiple-case outbreaks and the identification of nonpork sources of infection require ongoing education and control measures.

In other parts of the world, the infected-meat source statistics vary; in the former Soviet Union, >90% of the cases have been attributed to the ingestion of poorly cooked bear and wild boar meat. Factors contributing to the slow decline of trichinosis incidence in Russia and to the increase in the number of cases originating from wild-animal meat include the distribution and consumption of veterinary-uncontrolled pork, poaching and distribution of wild-animal meat, and poor compliance with regulations (28). Twenty-seven outbreaks of human disease occurred in China between 1964 and 2004 and were associated with mutton, dog, and game meat. However, the quarantine of infected meat is not mandatory in China (29). Although outbreaks are rare in Israel, outbreaks have been detected in immigrant agricultural workers; infected wild-boar meat was implicated (30). In tropical Africa, the infected meat source tends to be bush pigs and warthogs. Most infections in Central and South America have been associated with domestic pigs. It is difficult to say with certainty exactly which animals may be infected throughout the world; very few comprehensive studies of wild animals have been attempted. In one study of *Trichinella* infection in wildlife in the southwestern United States, the range of *T. murrelli* was extended from previous reports limiting this species to the eastern United States. Thus, *Trichinella* infection is now documented in three states bordering Mexico, New Mexico, Arizona, and Texas (31). Information is also now available confirming the presence of anti-

TABLE 14.6 Outbreaks due to consumption of *Trichinella* spp. in horse meat[a]

	Outbreak[b]					
Characteristic	**Italy, 1975 (*T. britovi*)**	**France, 1976 (*T. spiralis*, probably)**	**France, 1985 (*T. nativa*)**	**France, 1985 (*T. spiralis*)**	**Italy, 1986 (*T. britovi*)**	**France, 1993 (*T. spiralis*)[c]**
No. of cases	89	125	343	396	161	444
Percentage of patients with:						
Fever	+	65	90	85	70	81
Myalgia	+	59	93	88	67	82
Weakness		NE	87	77	NE	NE
Facial edema	+	57	58	84	62	75
Diarrhea	+	16	50	41	21	35
Vomiting		8	NE	NE	9	NE
Headache		60	58	51	66	NE
Rash		5	44	11	4	NE
Ocular involvement		31	28	34	26	NE
Mortality rate	0%	0%	0%	0.4%	0%	0%

[a] From reference 4; Ancelle T et al, *Am J Trop Med Hyg* **59:**615–619, 1998; and Bruschi F, *in* Sun T (ed), *Progress in Clinical Parasitology,* vol. 4, CRC Press, Inc., Boca Raton, FL.

[b] +, Symptoms present, actual patient numbers not provided; NE, not evaluated.

[c] Severe neurotrichinosis, 1.4%; cardiac symptoms or electrocardiograph changes, 4.7%.

Trichinella antibodies in a human population living in Papua New Guinea (32).

In Africa, *T. britovi* has been found in northern and western Africa, while *T. nelsoni* is found in the eastern part from Kenya to South Africa. Zoonotically, *T. britovi* is the second-most common species of *Trichinella* that affects human health. The host range for *T. nelsoni* includes sylvatic carnivores, bush pigs, and warthogs, some of which have been the source of human infections. Fewer than 100 human infections have been documented for this species in Kenya and Tanzania. However, an increase in the bushmeat trade and the creation of Transfrontier Conservation Areas (TFCAs) may have increased the risk of human trichinosis in the region. *Trichinella* T8 has been recovered in South Africa and Namibia (19). *T. zimbabwensis* has been detected only in wild and farmed reptiles of Africa (Zimbabwe, Mozambique, South Africa, and Ethiopia), al-

TABLE 14.7 Case definition for human trichinosis according to the European Center for Disease Control[a]

Criterion group	Prerequisites and case classification[b]
Clinical	At least three of the following six: fever; muscle soreness and pain; gastrointestinal symptoms; facial edema; eosinophilia; subconjunctival, subungual, and retinal hemorrhages
Laboratory	At least one of the following two laboratory tests: demonstration of *Trichinella* larvae in tissue obtained by muscle biopsy; demonstration of *Trichinella*-specific antibody response by indirect immunofluorescence, ELISA, or Western blot (i.e., seroconversion)
Epidemiological	At least one of the following three: consumption of laboratory-confirmed parasitized meat; consumption of potentially parasitized products from a laboratory-confirmed infected animal; epidemiological link to a laboratory-confirmed human case by exposure to the same common source

[a] Modified from reference 14 with permission of the publisher.

[b] Case classification is as follows: possible case, not applicable; probable case, any person meeting the clinical criteria and with an epidemiological link; confirmed case, any person meeting the laboratory criteria and with clinical criteria within the past 2 months (to be reported to the European Union level).

TABLE 14.8 Algorithm for diagnosing acute trichinosis in humans[a]

Group	Symptom
A	Fever, eyelid and/or facial edema, myalgia
B	Diarrhea, neurological signs, cardiological signs, conjunctivitis, subungual hemorrhages, cutaneous rash
C	Eosinophilia (>1,000 eosinophils/ml) and/or increased total IgE levels, increased levels of muscular enzymes
D	Positive serology (with a highly specific test), seroconversion, positive muscular biopsy

[a] Modified from reference 14 with permission of the publisher. The diagnosis is very unlikely with one symptom from group A or one from group B or C; suspected with one symptom from group A or two from group B and one from group C; probable with three symptoms from group A and one from group C; highly probable with three symptoms from group A and two from group C; and confirmed with three symptoms from group A, two from group C, and one from group D, or any of group A or B, one from group C, and one from group D.

TABLE 14.9 Differential diagnosis of trichinosis[a]

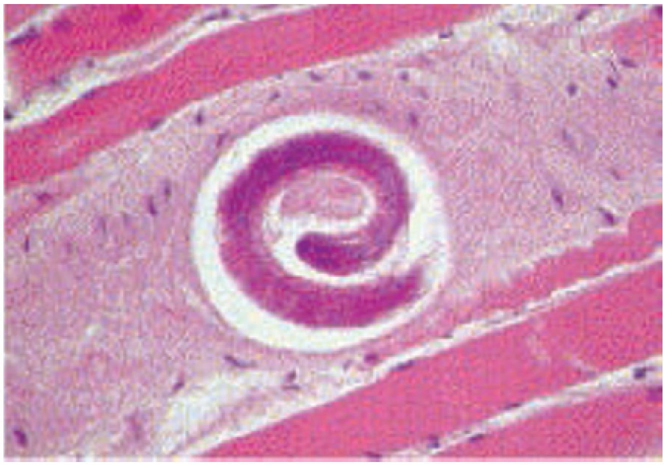
Clinical finding	Disease to be differentiated
Protracted diarrhea	Salmonellosis, shigellosis, and other viral, bacterial, or parasitic infections of the gastrointestinal tract
High fever and myalgia	Influenza virus infection
Periorbital or facial edema with fever	Glomerulonephritis, serum sickness, toxic-allergic reactions to drugs or allergens, polymyositis, periarteritis nodosa, dermatomyositis
High fever and neurological symptoms without periorbital edema	Typhoid fever
Intense headaches, fever, nuchal pseudorigidity with blurred consciousness and drowsiness, irritability, and neurological symptoms	Cerebrospinal meningitis, encephalitis, CNS infections
Intraconjunctival hemorrhages, intradermal petechiae, fever	Leptospirosis, bacterial endocarditis, and typhus exanthematicus
Eosinophilia with myalgia and an inflammatory response	Eosinophilia-myalgia syndromes (e.g., toxic oil syndrome, tryptophan intake, and eosinophilic fasciitis)
Eosinophilia with fever	Fasciolasis, toxocarosis, and invasive schistosomiasis

[a] Based on data from references 14, 17, and 19.

though experimentally it is able to infect mammals. Human infections are not known so far.

Although *T. spiralis* and *T. pseudospiralis* are found worldwide, the other species tend to have a more narrow geographic range (Table 14.2). *T. nativa* and *Trichinella* T6 are found primarily in the Arctic regions; *T. britovi*, *T. murrelli*, *Trichinella* T8, and *Trichinella* T9 are found in the temperate zones; and *T. nelsoni*, *T. papuae*, and *T. zimbabwensis* are found in equatorial areas.

Figure 14.5 Unencapsulated *Trichinella pseudospiralis* (image courtesy of Beck R et al, *Vet Parasitol* **159:**304–307, 2009). Note the absence of the collagen capsule seen with other species of *Trichinella*. doi:10.1128/9781555819002.ch14.f5



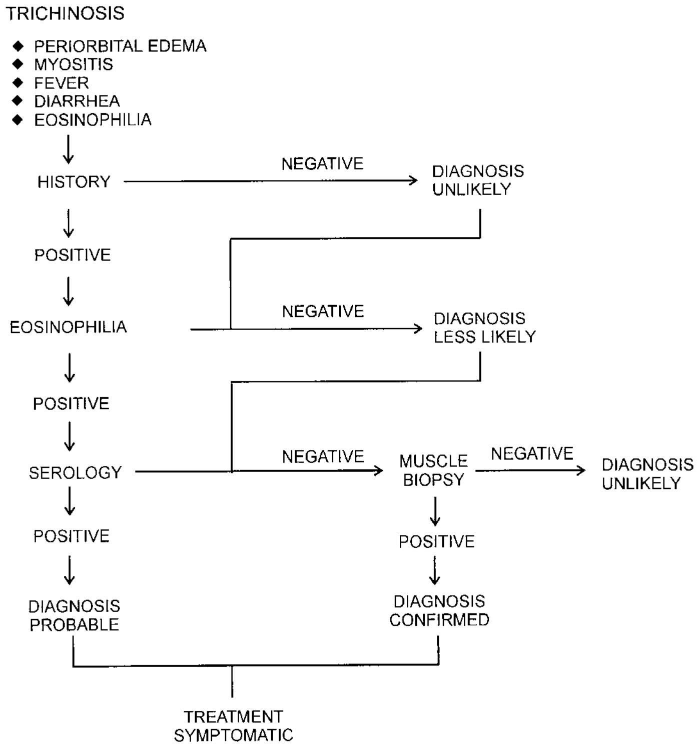
Countries in the European Union, Eastern European countries, and the former members of the Soviet Union require direct inspection of pork, using microscopic examination of small tissue samples of pig diaphragm or examination of pooled digested tissue samples; within the United States, the U.S. Department of Agriculture requires strict standards for the freezing, cooking, and curing of pork and pork products (33). A number of excellent preventive measures have been identified for implementation during the preparation of potentially infected meat sources, as well as control measures for commercial pig farms (Table 14.10). Recent information also confirms the need to review the intentional feeding of animal products and kitchen waste to horses, a high-risk practice which requires implementation of regulations to ensure that such feeds are rendered safe for horses, as is currently required for products fed to swine (34).

In 1981, the U.S. Department of Agriculture issued a news release that suggested that microwave cooking might not kill the larvae. On the basis of a number of subsequent studies, the current recommendation states that "*all parts* of pork muscle tissue must be heated to a temperature not lower than 137°F (58.3°C)" (4). It has been recommended that an internal meat thermometer be used when cooking pork; the meat can be tested after being removed from the microwave oven if the oven is not equipped with an internal thermometer. Reduction in the number of cases is due primarily to regulations requiring heat treatment of garbage and low-temperature storage of the meat. Occasional outbreaks are frequently due to problems with feeding, processing, and cooking of pigs raised for home use.

Baylisascaris procyonis

Although *Baylisascaris procyonis* was first isolated from raccoons in 1931 in the New York Zoological Park, it was also recognized in raccoons in Europe. The genus was defined in 1968 and was named after H. A. Baylis, who had been with the British Museum of Natural History in London.

B. procyonis was first recognized as causing neural larva migrans (NLM) in rodents and then recognized as being able to produce serious NLM in 100 different species of birds and mammals. The potential for causing human disease was considered by earlier parasitologists but has been recognized only during the last 30 years. Approximately 20 human cases have been documented and published, with >12 cases remaining unpublished (36). Raccoon roundworm encephalitis or *Baylisascaris* NLM is usually


TRICHINOSIS
◆ PERIORBITAL EDEMA
◆ MYOSITIS
◆ FEVER
◆ DIARRHEA
◆ EOSINOPHILIA
HISTORY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
EOSINOPHILIA
NEGATIVE
DIAGNOSIS LESS LIKELY
POSITIVE
SEROLOGY
NEGATIVE
MUSCLE BIOPSY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
POSITIVE
DIAGNOSIS PROBABLE
DIAGNOSIS CONFIRMED
TREATMENT SYMPTOMATIC

Algorithm 14.1 Diagnosis of trichinosis.
doi:10.1128/9781555819002.ch14.Alg14.1

associated with devastating neurologic outcome or death in children with the exception of a 4-year-old Louisiana boy with reported full recovery, an Oregon teenager who improved gradually, and a Canadian toddler who showed marked improvement (37).

Life Cycle and Morphology

B. procyonis is an ascarid normally found in raccoons (*Procyon lotor*), has a normal ascarid-like life cycle, causes a very serious zoonotic disease in humans, and is most often reported from North America (Fig. 14.6) (38). Raccoons are infected by ingesting infective eggs and by eating larvae encysted in the tissue of intermediate hosts, such as rodents, rabbits, and birds. The larvae then penetrate the mucosa of the small intestine and develop there before reentering the intestinal lumen to mature. Raccoons may be infected by up to 60 worms or more, and young animals have a higher prevalence of infection. These adult worms produce 150,000 eggs/worm/day; infected raccoons can shed as many as 250,000 eggs/g of feces. This level of egg production can lead to significant environmental contamination.

B. procyonis eggs are somewhat oval, are dark brown, and measure from 63 to 88 μm by 50 to 70 μm. The eggs contain a single-celled embryo and a thick shell with a finely granular surface; they are not infective immediately after being passed but can survive in moist soil for years (Fig. 14.7). *Toxocara* eggs tend to be somewhat larger and have a coarsely pitted thick shell.

Human infections result from ingestion of eggs that are passed in very large numbers (millions of eggs per day) in the feces of infected raccoons; the human then becomes the accidental intermediate host. Once ingested, the eggs hatch in the intestinal tract, releasing the immature larvae. Rather than developing to adult worms as occurs in the raccoon, the larvae begin to migrate extensively throughout the body tissues, causing visceral larva migrans (VLM) and/or NLM. Although this infection presents as acute fulminant eosinophilic meningoencephalitis, two features of the life cycle are somewhat different from those of other helminths causing larva migrans: there is targeted migration to the CNS and continued growth of the larvae to a much larger size within the CNS. Despite different courses of therapy, there are very rare documented neurologically intact survivors of this infection.

Clinical Disease

The first confirmed cases of human *B. procyonis* infection were described in the 1980s. Risk factors have been identified as contact with infected raccoons, their feces, or a contaminated environment. Geophagia or pica, which is often seen in children younger than 2 years, has also been identified as a potential risk. Tissue damage is caused by the actual larval migration, as well as an intense inflammatory reaction (Fig. 14.8 and 14.9). Unlike other helminth larvae

TABLE 14.10 Trichinosis: prevention and control measures

Potential infection source (reference)	Prevention and control measures
Meat preparation (4)	1. Cook meat products until the juices run clear or to an internal temperature of 160°F (71°C). 2. Freeze pork less than 6 in. thick for 20 days at 5°F (–17°C) to kill any encysted larvae. Other options would be –10°F (–23°C) for 10 days or –20°F (–29°C) for 6 days. However, remember that *T. nativa* in bear meat probably survives freezing for a year or longer. 3. Cook wild game meat thoroughly. Freezing wild game meats, unlike freezing pork, even for long periods, may not effectively kill all encysted larvae. 4. Clean meat grinders after preparing ground meats. 5. Curing (salting), drying, smoking, or microwaving meat does not consistently kill infective larvae.
Pig farms (10)	1. Barriers must be in place to prevent entrance of rodents and other potential hosts into the pigsty and food store. 2. New animals should not be admitted to the farm prior to serologic testing for antibodies to *Trichinella* spp.[a] 3. Procedures for sanitary disposal of dead animals must be used at all times. 4. No raw or improperly heated swill or waste food containing meat may be present at the farm. 5. No rubbish dumps should be located in the immediate vicinity of the farm.

[a] The use of serologic tests has been an important technological advance, not only for the diagnosis of human infection but also for the identification of infected animals (10).

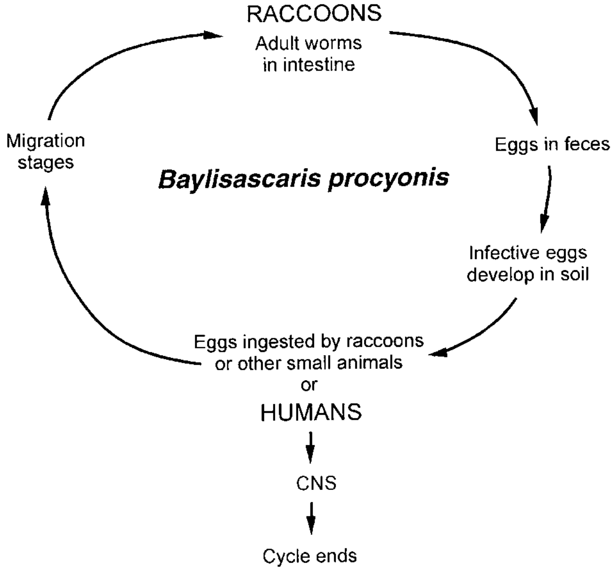
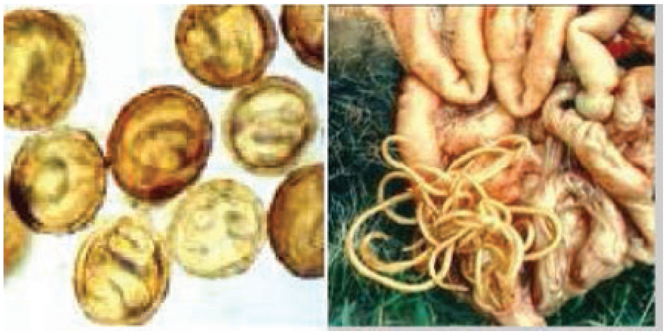


Figure 14.6 Life cycle of *Baylisascaris procyonis*. Accidental infection in the human leads to visceral larva migrans (VLM), ocular larva migrans (OLM), and/or neural larva migrans (NLM), which are very serious diseases that can cause death. Infections are seen primarily in very young children. doi:10.1128/9781555819002.ch14.f6

that cause VLM, the larvae of *B. procyonis* continue to grow during the migratory phase of the life cycle and can reach lengths of 2 mm. In addition to continued growth, the larvae tend to exhibit very vigorous migratory behavior and remain viable for long periods. They tend to invade the eyes, causing ocular larva migrans (OLM), which has also been found in immunocompetent adults, and the spinal cord and brain, causing NLM, found primarily in infants and young children. Unfortunately, the relatively small brain size in infants or small children increases the potential that larval migration will cause severe clinical disease, including permanent neurologic damage, blindness, or death. It is also important to realize that, with the absence of a definitive diagnostic test, the prevalence of subclinical cases is totally unknown; it is probably higher than is currently recognized. It is very likely that asymptomatic infection also occurs (38). Unfortunately, even with clinical cases, most patients are diagnosed only after there has already been severe CNS damage.

Patients can present with eosinophilic meningoencephalitis or unilateral neuroretinitis; the presentation probably depends on the number of eggs that were ingested. Larvae tend to enter the CNS approximately 1 to 4 weeks after infection, and the disease may progress very rapidly. Numerous granulomata have been seen in the heart, mediastinal soft tissues, pleura and lungs, small and large bowel walls, and mesentery and mesenteric lymph nodes (38). Nonspecific manifestations of this larval migration include

Figure 14.7 (Left) *Baylisascaris procyonis* eggs; note the larvae within the egg shells (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). (Right) Adult worms from raccoon intestine (courtesy of CDC Public Health Image Library). doi:10.1128/9781555819002.ch14.f7



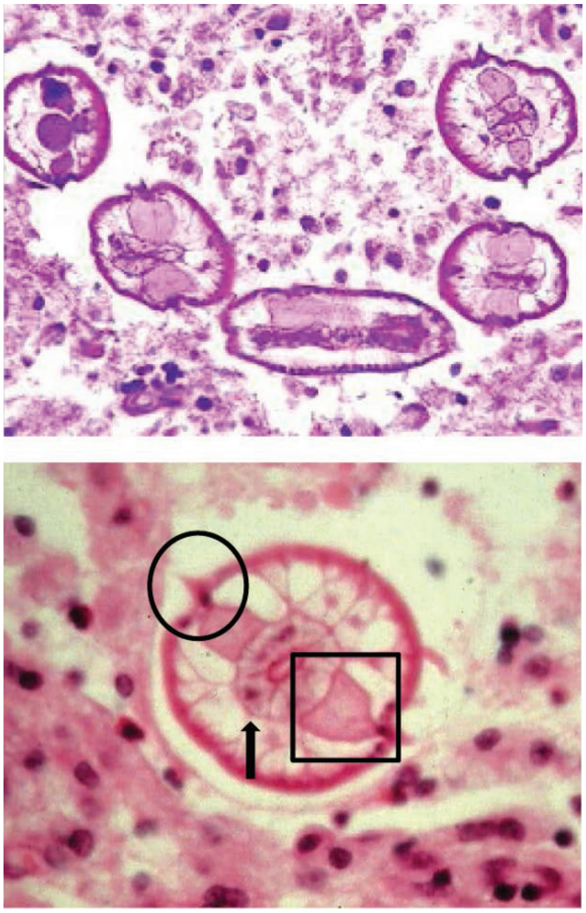


Figure 14.8 *Baylisascaris procyonis*, CNS atrophy, sclerosis, and larval granuloma in the brain of a 2-year-old boy who died following 14 months of CNS problems. (Lower) Note the prominent alae (circle), excretory columns (box), and multinuclear intestinal cells (arrow). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of K. R. Kazacos.)
doi:10.1128/9781555819002.ch14.f8

a macular rash primarily on the face and trunk, pneumonitis, and hepatomegaly (38). The development of dyspnea and tachypnea appears to be secondary to early pulmonary migration.

OLM may present with a broad range of symptoms, including chronic endophthalmitis with retinal detachment, posterior pole granuloma, vitreous abscess, pars planitis, optic neuritis, keratitis, uveitis, iritis, hypopyon, and meandering retinal tracks containing larvae. A new clinical entity, diffuse unilateral subacute neuroretinitis (DUSN), is also caused by *B. procyonis* (39). DUSN is a form of OLM characterized by progressive unilateral visual loss, retinal pigmentation, and optic nerve anatomy changes, all of which lead to severe ocular damage.

Massive larval invasion of the CNS is characteristic, with estimates of more than 3,200 larvae being isolated from the brain of a single case (3 larvae/g of tissue) (40). This neural form, NLM, may present with symptoms ranging from mild neuropsychologic problems to seizure, convulsions, ataxia, coma, and death. Patients may exhibit sudden lethargy, irritability, loss of muscle coordination, decreased head control, spasmodic contractions of the neck muscles, stupor, nystagmus, obtundation, coma, hypotonia, and hyperreflexia. Infants who have survived meningoencephalitis demonstrate sequelae including hemiparesis, inability to sit or stand, ocular muscle paralysis, cortical blindness, and severely delayed development. Seizures often occur, and these symptoms tend to be difficult to control. The patients may deteriorate rapidly, progressing

Figure 14.9 Neuroimaging of human brain with *B. procyonis* NLM. (A) In acute NLM, an axial-flair magnetic resonance (MR) image (at the level of the posterior fossa) demonstrates an abnormal hyperintense signal of cerebellar white matter; (B) an axial T2-weighted MR image (at the level of the lateral ventricles) demonstrates an abnormal patchy hyperintense signal of periventricular white matter and basal ganglia; (C) an axial T2-weighted MR image (at the level of the lateral ventricles) of a patient with subacute/chronic NLM demonstrates residual abnormal hyperintense signal of the periventricular white matter, loss of white matter volume, and dilation of ventricles and sulci, consistent with generalized cerebral atrophy. (Images courtesy of Gavin PJ et al, *Clin Microbiol Rev* **18:**703–718, 2005.)
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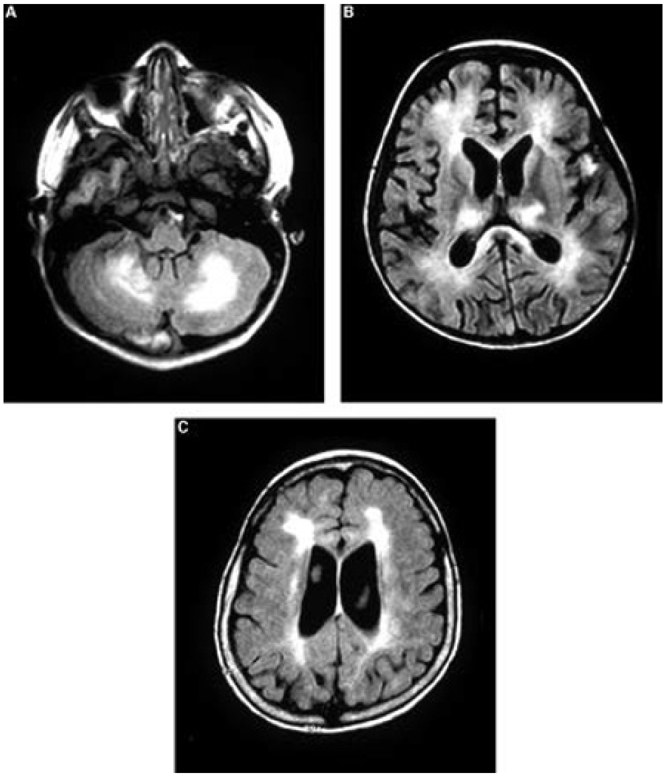


to stupor, coma, and death. Survivors are left in a persistent vegetative state or with severe neurologic deficits, including blindness, all of which can require extensive supportive care.

Diagnosis

Human infections with *B. procyonis* are rare and are often diagnosed by a process of elimination; when all other recognized causes of larva migrans have been explored, *B. procyonis* may be considered (Table 14.11). There are approximately 20 documented cases, almost all of which have been seen in young children. Results obtained from routine hematologic and CSF examinations are usually consistent with a parasitic infection but are nonspecific. Definitive diagnosis requires identification of the larvae in tissues; however, this can be difficult depending on the body site. Cross sections of larvae tend to measure 60 to 70 µm, and the larvae have prominent, single lateral alae and paired, conical excretory columns (smaller than central intestine) (Fig. 14.8). Ocular examinations may reveal retinal lesions, larval tracks, or migrating larvae; these findings may provide the tentative diagnosis of a helminth infection.

Using IFAT, ELISA, and Western blotting, anti-*Baylisascaris* antibodies can be detected in CSF or serum. Currently, the source of serologic testing is the CDC (Table 14.11). Acute- and convalescent-phase titers demonstrate several-fold increases in both serum and CSF antibody levels; there is no cross-reactivity with *Toxocara*. With the availability of a reliable serologic test, there is less need to perform a brain biopsy. *In most cases, the clinical history provides the main clues.* In the absence of large population-based serologic studies, *Baylisascaris* seroprevalence remains unknown. However, the detection of anti-*Baylisascaris* antibodies in asymptomatic family members of documented human cases, in individuals who have had contact with raccoons, and preliminary

TABLE 14.11 Baylisascariasis: clinical presentations and diagnosis

Type	Description	Diagnosis
Visceral larva migrans	Association with macular rash, abdominal pain, hepatomegaly, and pneumonitis. Larvae can cause inflammatory reactions in organs and tissue damage. Skin infection and inflammation have been reported.	Findings include peripheral eosinophilia, deep white matter abnormalities on MRI (changes may lag behind clinical disease), and positive *Baylisascaris* antibody titers from CSF and serum. Neuroimaging and encephalography may provide supportive evidence and/or confirmation of neural larva migrans. Ocular examinations may reveal a migrating larva, larval tracks, or lesions consistent with presence of a nematode larva in the eye.
Ocular larva migrans	Unilateral subacute neuroretinitis, photophobia, retinitis, and/or blindness (typically unilateral). Eye disease may or may not be present.	Examination of tissue biopsies and morphological identification of larvae may be helpful, but in cases with a low larval load, it may be difficult to obtain a specimen containing a larval cross section required for confirmation.
Neural larva migrans	Often presents as acute eosinophilic meningoencephalitis. Symptoms develop within 2 to 4 weeks after ingestion of large numbers of infective eggs and include weakness, incoordination, ataxia, irritability, weakness, seizures, altered mental status, stupor, and/or coma. Once symptoms and signs of neurologic disease are detected, significant pathology usually is already present.	In the absence of a brain biopsy, the diagnosis may depend on serologic testing. There is no commercially available serological test for baylisascariasis. However, in highly suspicious cases, CSF or serum can be submitted to CDC for antibody testing. If the *Baylisascaris* serology is negative, serological testing for other nematode infections should be considered and may help to diagnose other causes of larva migrans. Please contact CDC for more information about baylisascariasis testing. CDC information: 1-800-232-4636 Health care professionals: 1-404-718-4745 After-hours emergencies: 1-770-488-7100

Comments

Other parasitic nematode infections may cause similar signs and symptoms, including those caused by migrating larvae of *Toxocara* spp. and *Angiostrongylus* spp. Baylisascariasis is similar to that caused by *Toxocara* spp. However, disease progression is more severe because, unlike *Toxocara*, *Baylisascaris* larvae are larger and continue to molt and increase in size, thus resulting in extensive damage in the CNS, heart, and other tissues.

Baylisascaris infection should be considered in individuals (particularly young children) who present with severe developmental disabilities or a history of pica/geophagia and sudden onset of eosinophilic encephalitis. A history of exposure to raccoons or their feces is highly suggestive *but not necessary.*

TABLE 14.12 Differential diagnosis of *Baylisascaris procyonis* infection

Infection	Geographic areas	Clinical findings	Comments
Baylisascaris procyonis	Americas from Canada to Panama, Europe, Japan, Soviet Union, Asia	Peripheral and CSF eosinophilia, meningoencephalitis	Eosinophilic meningoencephalitis; history of pica or geophagia, age of patient extremely important in suspecting infection
Toxocara spp.	Cosmopolitan	VLM	Uncommon cause of NLM; antibodies in blood and CSF
Angiostrongylus cantonensis	Southeast Asia, China, Japan, Jamaica, Western Pacific Islands, Hawaii, Madagascar, Cuba, Egypt, Puerto Rico, New Orleans, Nigeria, eastern Australia, Africa	Eosinophilic meningitis	Relatively benign course, usually good prognosis
Gnathostoma spinigerum	Southeast Asia, China, Japan	Severe neurologic sequelae, myeloencephalitis, focal cerebral hemorrhage (with xanthochromia), radiculopathy, migrating cutaneous swellings	Poor prognosis
Less common causes of eosinophilic meningoencephalitis in humans Neurocysticercosis Paragonimiasis Toxocariasis Neurotrichinosis Schistosomiasis	Cosmopolitan in many areas; more narrow geographic areas would apply to schistosomiasis	Could mimic infection with *B. procyonis*	History critical in suspecting any of these infections
Coccidioides immitis	Southwestern United States, Central and South America	Intense basilar enhancement, hydrocephalus, acute infarction on neuroimaging, positive CSF serology	Disseminated disease, most common cause of eosinophilic meningitis in United States
Acute disseminated encephalomyelitis	Cosmopolitan	Acute encephalopathy, cerebral white matter changes on neuroimaging	Monophasic, nonprogressive illness, more discrete multifocal gray and white matter abnormalities on neuroimaging; generally good prognosis

results of a seroprevalence study in Chicago area children suggest that low-level asymptomatic infection may occur (72).

The liver and lungs do not tend to be involved in *Baylisascaris* infections, but cerebral lesions are often detected. Well-formed granulomas can be seen in any part of the nervous system. The damage tends to be prominent, even showing tracks with tissue disruption. Differential diagnosis findings are summarized in Table 14.12.

Treatment

There is no effective cure for *B. procyonis* infection; treatment is symptomatic and involves systemic corticosteroids and anthelmintic agents. Unfortunately, NLM is usually not responsive to anthelmintic therapy; by the time the diagnosis is made, extensive damage has already taken place. Drugs that can be tried include albendazole, fenbendazole, mebendazole, thiabendazole, tetramisole, levamisole, diethylcarbamazine, and ivermectin. Experimental data from animal studies indicate that albendazole and diethylcarbamazine may have the best CSF penetration and larvicidal activity. Considering these two drugs, only albendazole has been used in children with NLM, and has better pharmacologic properties (absorption, high serum concentrations, good blood-brain barrier penetration, and low toxicity). Unfortunately, since the diagnosis does not occur until the onset of symptoms, larval invasion of the CNS has already taken place. Therefore, treatment is started late in the course of the infection.

Considering the potential outcomes associated with delaying treatment until symptoms begin, prophylactic treatment for asymptomatic children exposed to raccoons or contaminated environments is now being considered. Since the anthelmintics do not appear to be problematic, prophylaxis seems appropriate for specific individuals. Albendazole has been used in these cases; treatment has been discontinued when environmental testing has proven to be negative.

Ocular infections have been treated successfully by using laser photocoagulation therapy to destroy the intraretinal larvae (41). Systemic corticosteroids have been used to decrease or prevent resulting intraocular inflammatory responses from the killed larvae.

Epidemiology and Prevention

The relationship between *B. procyonis* infection, raccoons, and humans has now been well defined. Recent reports of patent *B. procyonis* infections in domestic dogs and puppies have caused concern because of the potential for expanded human exposure to infective eggs in feces in the absence of raccoons (42). Groups of raccoons tend to defecate in common areas called latrines, which tend to be present off the ground in fallen logs (firewood may be contaminated), rocky outcroppings, and trees. In areas that have been carefully investigated such as Pacific Grove, CA, many latrines are present and are located directly on the ground, on roofs, in attics, and on steps and fences. These findings suggest that a very large number of raccoons are present in this location. The eggs remain viable in the soil for extended periods, often years. The eggs also have a sticky surface coating that causes them to adhere to objects, including human hands and toys. Apparently, incineration or soaking the feces with volatile solvents such as mixtures of xylene and ethanol appears to be the only means of killing the eggs (38). In some cases, removal and disposal of several inches of topsoil may also be indicated. Chemical disinfection is rarely effective and not practical for large outdoor areas. Eggs are resistant to most common disinfectants; 20% bleach (1% sodium hypochlorite) will wash away sticky eggs but does not kill them. Recognition of this new human infection and prevention of the establishment of raccoon latrine sites around areas of human habitation and recreational use are critical to successful control.

This infection is of great public health concern and has the potential to cause extensive damage in the human host, particularly young children (38). Risk is highest for young children or infants with pica or geophagia; these individuals need to be kept away from potentially contaminated areas. Parents and care givers should stress the importance of hand washing after outdoor play or contact with animals, including pet dogs. Raccoons should be discouraged from visiting yards by refraining from putting out food and by not leaving dog food uncovered and available. Keeping pet raccoons, particularly in homes with young children, should be discouraged.

Although *B. procyonis* was thought to be absent from many regions, it is now becoming clear that where raccoons are found *B. procyonis* is also likely to be present, and these animals have been introduced worldwide (38). The infection has also been found in a 3-day-old domestic lamb, suggesting that mammalian fetuses, in general, should be added to the list of those at risk for NLM due to ascarid parasites (43). The increasing number and recognition of cases highlight the critical importance of controlling and preventing this potentially devastating zoonotic infection (44). Certainly education for the public will be paramount in helping to determine at-risk populations and preventing additional infections.

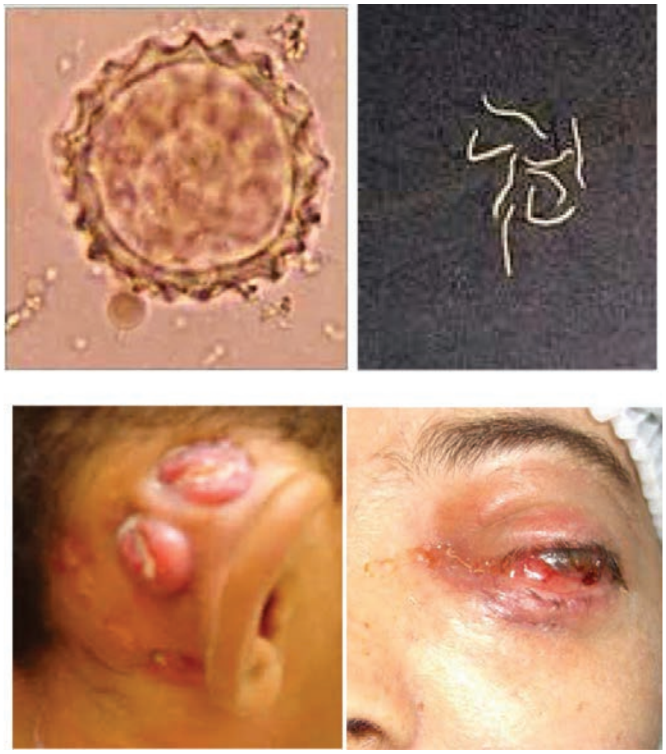
Lagochilascaris minor

Although human infections with *Lagochilascaris minor*, which is normally found in the small intestine of the cloudy leopard, have been documented, neither the natural life cycle nor the route of human infection is known (45). Human cases have been recorded from the West Indies, Suriname, Costa Rica, Mexico, Venezuela, Colombia, Paraguay, and Brazil. The cases of human infection are characterized by lesions in the oropharynx and other soft tissues in the head and neck. The first sign of infection may be a pustule, usually on the neck, which increases in size to a large swelling. After the skin breaks, living adults, larvae, and eggs are expelled at intervals in the pus. Eggs are continually developing into larvae and then into adults in the tissues at the base of the abscess; thus, the life cycle is a continuing process. Adult male worms measure up to 9 mm long, and the females are about 1.5 cm; the eggs resemble those of *Toxocara cati* and measure 45 to 65 μm by 59 to 73 μm. Irregularly shaped pits are present on the egg surface; 20 to 32 pits surround the equator of an egg (Fig. 14.10). The area of induration increases, with the development of sinus tracts. Chronic granulomatous inflammation may last for months. Other tissues that have been involved include the throat, nasal sinuses, tonsillar tissue and mastoids, brain, and lungs. Although thiabendazole has been tried, efficacy was not really documented. Albendazole has also been tried, but it was possible to confirm only transitory elimination of adult worms.

A study in which wild rodents were used as experimental intermediate hosts of *L. minor* has provided some additional information about the life cycle and epidemiology of this parasite (46). After inoculation of infective eggs, larvae were found in viscera, skeletal muscle, and adipose and subcutaneous tissues from all rodents. Adult worms were recovered in the cervical region, rhinopharynx, and oropharynx of domestic cats fed the rodent tissues. Based on this study, it appears that (i) wild rodents act as intermediate hosts, (ii) under natural conditions rodents could act as either intermediate hosts or paratenic hosts of *L. minor*, (iii) despite the occurrence of an autoinfection cycle in felines (definitive hosts), the cycle is completed only when intermediate hosts are provided, and (iv) in the wild, rodents could serve as a source of infection for humans since they are frequently used as food (guinea pigs and agoutis as examples) in regions with the highest incidence of human lagochilascariasis. Thus, humans are accidental hosts, possibly becoming infected by eating raw meat from wild rodents containing L3 larvae. It is quite possible that autoinfection also occurs; this possibility is suggested by the chronicity of the disease in patients over a number of years.

L. minor lesions in animals and humans characteristically result in tumors and fistulas with cutaneous and subcutaneous abscesses localized in the cervical region and surrounding tissues. However, parasite lesions of the mastoids, jaw, tonsils, maxillary and paranasal sinuses, middle ear, oropharynx, pharynx, dental alveoli, and CNS have been observed (45). Unfortunately, several patients reported having received multiple ineffective treatments as their lesions were misdiagnosed as bacterial infections. Adequate diagnosis and prompt treatment of suspected human lagochilascariasis are required. A differential diagnosis must consider paracoccidioidomycosis, tuberculosis, actinomycosis, and leishmaniasis (1).

Figure 14.10 (Upper left) *Lagochilascaris minor* egg; note the pits in the egg shell (somewhat similar to *Toxocara* eggs); (right) adult worms removed from a human lesion. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) (Lower left) Ulcerating lesions behind the ear in a case of lagochilascariasis (courtesy of Neto FXP et al, *Rev Brasil Otorrinolaringol*, suppl 73, 2007); (right) toxocariasis with eye involvement (courtesy of Yoshimi R et al, *Rev Brasil Otorrinolaringol*, suppl 72, 2006).
doi:10.1128/9781555819002.ch14.f10



Toxocara canis and *T. cati* (Visceral Larva Migrans and Ocular Larva Migrans)

The VLM syndrome was described by Beaver and colleagues in New Orleans in 1952, while analyzing liver biopsies taken from three children suffering from hepatomegaly, respiratory symptoms, anemia, and a highly elevated eosinophilia. This syndrome is caused by the migration of larvae of *Toxocara canis*, *T. cati*, and some other animal helminths. Within 10 years of the initial report, more than 2,000 cases had been reported from 48 countries and from every region of the United States. The disease, frequently seen in young children, usually does not cause severe problems, although it persists for months to more than a year. One serious possible complication is invasion of the eye (OLM), often resulting in a granulomatous reaction in the retina. Larva migrans caused by *Toxocara* spp. is widely recognized as a zoonotic infection throughout the world and may be much more common than previously thought. Infection rates in dogs have been reported to be 2 to 90%, and the highest rates are seen in puppies as a result of transmission from their dams. The overall incidence of infected dogs older than 6 months is probably less than 10% (47).

Life Cycle and Morphology

Humans acquire the infection by ingesting infective eggs of the dog (primarily) or cat ascarid, *T. canis* or *T. cati* (Fig. 14.11 and 14.12). Puppies are often infected by vertical transfer of larvae from their dams transplacentally or lactogenically, and egg shedding by puppies can begin as early as 2 weeks of age. In cats, lactogenic but not transplacental transmission occurs. Young kittens and puppies tend to recover from the infections between 3 and 6 months of age. Infections in older animals are acquired by the ingestion of infective eggs from the soil or ingestion of larvae in infected rodents, birds, or other paratenic hosts. Eggs are shed in the feces and take about 2 to 3 weeks to mature and become infective. After the eggs are accidentally ingested by a human, the larvae hatch in the small intestine, penetrating the intestinal mucosa and migrating to the liver. Migratory routes include the lungs and/or other parts of the body, or the larvae may remain in the liver. During this migration, the larvae do not mature, even if they make their way back into the intestine. The larvae are usually 0.5 mm long and 20 μm wide. Information also implicates the ingestion of uncooked meats as a potential cause of human toxocariasis, with possibly the first North American case, following ingestion of raw lamb liver, being reported by Salem and Schantz (48). Although transplacental

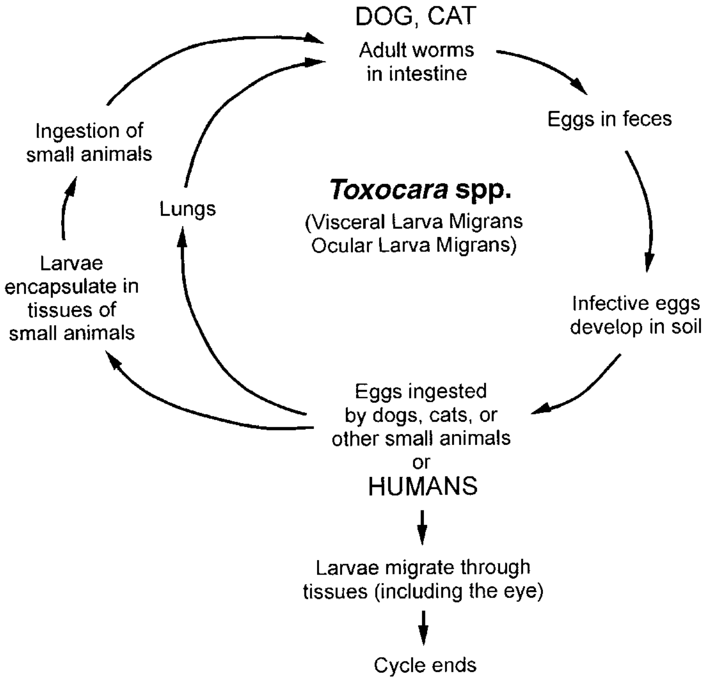
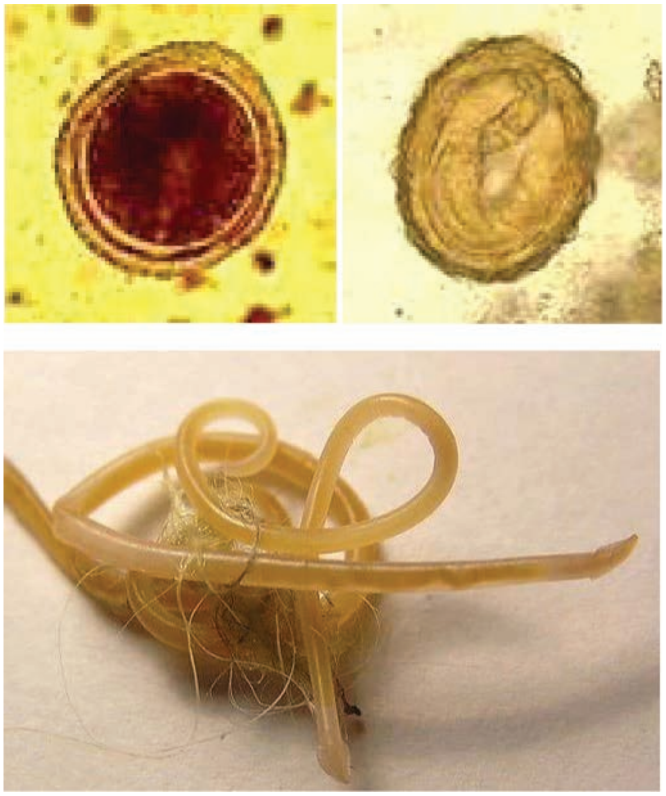

DOG, CAT
Adult worms in intestine
Eggs in feces
Toxocara spp.
(Visceral Larva Migrans Ocular Larva Migrans)
Infective eggs develop in soil
Eggs ingested by dogs, cats, or other small animals or
HUMANS
Larvae migrate through tissues (including the eye)
Cycle ends
Lungs
Ingestion of small animals
Larvae encapsulate in tissues of small animals

Figure 14.11 Life cycle of *Toxocara* spp., the cause of visceral larva migrans (VLM) and ocular larva migrans (OLM). doi:10.1128/9781555819002.ch14.f11

Figure 14.12 *Toxocara canis*. (Upper left) Immature egg; (right) mature egg containing larva. (Lower) Adult worms. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch14.f12



and lactogenic transmission has not been reported to occur in humans, in a study of *Toxocara* titers in maternal and cord blood, 6 (35%) of 17 mothers in the *Toxocara* antibody-positive group had previously miscarried compared with 3 (8.6%) of 35 *Toxocara*-negative mothers (49).

Most infections are probably asymptomatic and/or go unrecognized. VLM tends to be seen in younger children, around the age of 3 years, whereas OLM is more likely to occur in older children, around 8 years. However, this does not tend to be the case with *B. procyonis*, where most cases of VLM, NLM, and OLM have been identified in very young children.

Although VLM is generally associated with *T. canis* or *T. cati*, other helminths have also been implicated in disease. *B. procyonis* has been implicated in VLM, NLM, and OLM in humans. An excellent review of the diagnostic morphology of four larval ascaridoid nematodes that may cause VLM includes identification keys for *Toxascaris leonina*, *B. procyonis*, *Lagochilascaris sprenti*, and *Hexametra leidyi* (50). If discovered in tissue sections, the four species of ascaridoid larvae described in the study by Bowman (50) can be differentiated from other known ascaridoids that may cause VLM.

Clinical Disease

Clinical symptoms depend on the number of migrating larvae and the tissue or tissues involved. Infections may range from asymptomatic to severe disease. There are two defined clinical syndromes resulting from infection. VLM occurs most commonly in young children and results in hepatitis and pneumonitis as the larvae migrate through the liver and lungs, respectively. OLM occurs more frequently in older children and adolescents and may result from the migration of even a single larva in the eye (51–54). The most outstanding feature of the disease is a high peripheral eosinophilia, which may reach 90%. The overall severity of the clinical picture depends on the initial dose of infective eggs. As few as 200 *T. canis* larvae in small children may produce a peripheral eosinophilia of 20 to 40% for more than a year, with no other detectable symptoms. Patients with 50% eosinophilia usually have symptoms, which might include fever, hepatomegaly, hyperglobulinemia, pulmonary infiltrates, cough, neurologic disturbances, and endophthalmitis. Although a rare complication of toxocariasis, CNS involvement can cause seizures, neuropsychiatric symptoms, or encephalopathy (51, 52).

VLM Syndrome. The VLM syndrome is most commonly seen in children 1 to 4 years of age; however, rare cases have been seen in adults, in whom the illness includes mild pulmonary involvement suggestive of VLM. Severe bronchospasm resulting in respiratory failure was not reported until 1992; the case was confirmed by ELISA (53). The full clinical presentation includes hepatomegaly and pulmonary infiltrates or nodules accompanied by cough, wheezing, eosinophilia, lymphadenopathy, and fever. Larval entry into the CNS can also result in meningoencephalitis and cerebritis, manifesting as seizures. Larvae often remain in the liver and/or lungs, where they become encapsulated in dense fibrous tissue. Other larvae may continue to migrate throughout the body, causing inflammation and granuloma formation

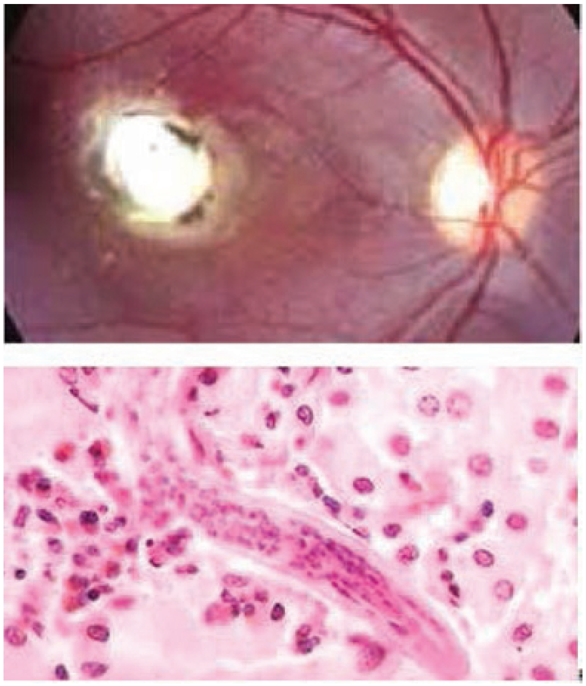
A report from Mexico City reviewed six adult patients with toxocariasis presenting with rheumatic symptoms, including lower-extremity nodules, edema suggestive of thrombophlebitis, and synovitis of a knee without effusion. Some of the patients reported having a prolonged, nonproductive cough, generalized pruritus, and migratory cutaneous lesions. One patient with monarthritis subsequently developed orchitis. All patients had an eosinophilia of 14 to 20%. One patient was biopsy positive, two had positive serologic test results, and the diagnosis was never confirmed in the other three (55). In another case, a 17-year-old boy with palpable purpura, oligoarthritis, acute abdominal pain, microhematuria, and cutaneous vasculitis was found to have toxocariasis with a clinical history including hypereosinophilia and domestic contact with a puppy (56). The infection was confirmed using serologic tests, and complete spontaneous resolution occurred within a few days.

The relationship between asthma and covert toxocariasis remains unclear; however, in a 1999 study, the seroprevalence of anti-*T. canis* antigen (E/S antigen) was 26.3% in asthmatic patients and 4.5% in the controls. All asthmatic patients with anti-*Toxocara* immunoglobulin E (IgE) had cutaneous reactivity to E/S antigen. Therefore, the authors concluded that asthmatic patients with anti-*Toxocara* IgE and IgG were experiencing a covert toxocariasis (57). Significantly elevated levels of IgE/anti-IgE immune complexes have been detected in sera of patients with symptomatic disease, including VLM and OLM (58). While specific IgG may act via antibody-dependent cell-mediated cytotoxicity mechanisms, IgE/anti-IgE immune complexes may participate in VLM and OLM by inducing type III hypersensitivity.

OLM Syndrome. Evidence suggests that ocular disease can occur in the absence of systemic involvement and vice versa for VLM. Although these facts may be explained by possible strain differences of *Toxocara* spp., VLM may reflect the consequences of the host inflammatory response to waves of migrating larvae while OLM may occur in individuals who have not become sensitized (59). The resulting inflammation presents clinically as either a granuloma or a granulomatous larval track in the retina or as a condition of the vitreous that resembles endophthalmitis (Fig. 14.13).

Patients with symptoms that do not fall into the more strict categories of VLM or OLM are often described as

Figure 14.13 (Upper) Ocular larva migrans (toxocariasis); note the white, elevated granuloma on the retina. (Courtesy of Sowka JW et al, *Handbook of Ocular Disease Management. Toxocariasis: Ocular Larva Migrans.* Jobson Medical Information LLC, New York, NY, 2005.) (Lower) *Toxocara* larva recovered in liver (visceral larva migrans). doi:10.1128/9781555819002.ch14.f13

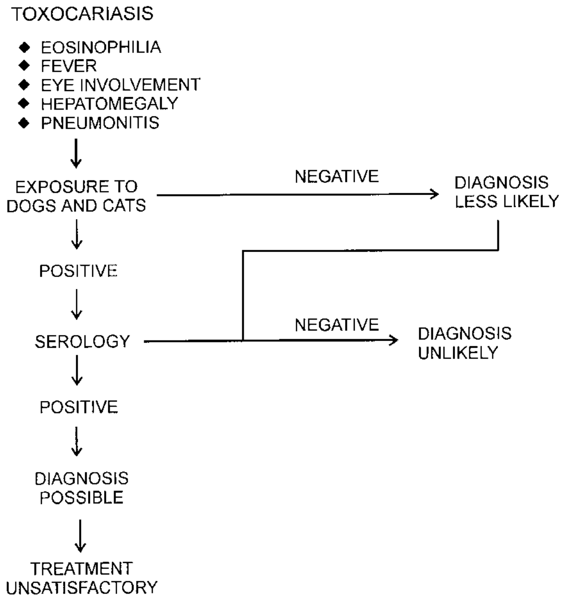


having covert toxocariasis. These cases are characterized by symptoms including abdominal pain, anorexia, behavior disturbances, cervical adenitis, wheezing, limb pains, and fever (47).

Diagnosis

VLM symptoms caused by *Toxocara* spp. must be differentiated from those caused by other tissue-migrating helminths (ascarids, hookworm, filariae, *Strongyloides* spp., and *Trichinella* spp.), as well as other hypereosinophilic syndromes. OLM may be confused with retinoblastoma, ocular tumors, developmental anomalies, exudative retinitis, trauma, and other childhood eye problems. It is important to remember that in OLM peripheral eosinophilia may be absent. OLM should be considered in any child with unilateral vision loss and strabismus who has raised, unilateral, whitish, or gray lesions in the fundus. VLM should be suspected in any pediatric patient with an unexplained febrile illness and eosinophilia. If the patient has a history of pica and there is hepatosplenomegaly and multisystem disease, then VLM becomes even more likely.

The suspected diagnosis can be confirmed only by identification of larvae in autopsy or biopsy specimens. However, if children are found to have *Ascaris* or *Trichuris* infections, one might suspect toxocariasis, since all three infections are transmitted via ingestion of contaminated soil. Since biopsy specimens are usually not recommended, serologic testing has become widely accepted as the most appropriate approach. In patients with presumptive ocular toxocariasis, higher antibody titers have been detected in the aqueous humor than in the serum, suggesting localized antibody production. The EIA using *T. canis* excretory-secretory antigens from infective-stage larvae is the most useful diagnostic test for toxocaral VLM and OLM and is the assay used by most commercial reference laboratories in the United States, including the reference laboratory at CDC. This EIA is highly recommended and highly specific, and there are no cross-reactions with sera obtained from patients infected with other commonly occurring human parasites (54). The diagnostic titers vary between VLM throughout the body and OLM. A titer of 1:32 is considered diagnostic for VLM, while a titer of 1:8 is considered diagnostic for OLM. This lower titer for OLM raises the possibility of a false-positive diagnosis in a patient who has an asymptomatic *Toxocara* infection and an ocular disease due to other etiology. Measurement of antibody levels in ocular fluid should increase the specificity of the ELISA, yielding a better definitive diagnosis for the patient. The serologic tests become very important when one is trying to differentiate OLM from retinoblastoma, which may have serious consequences. Serologic testing for toxocariasis is recommended in patients with Fuchs heterochromic cyclitis and retinal scars in the absence of toxoplasmosis (60). Refer to Algorithm 14.2 for a detailed review. Although the currently recommended serologic test for toxocariasis is EIA, when interpreting serologic results a measurable titer does not always represent current infection. A small percentage of the U.S. population (2.8%) exhibits a positive titer that reflects the prevalence of asymptomatic toxocariasis.



Algorithm 14.2 Diagnosis of toxocariasis. doi:10.1128/9781555819002.ch14.Alg14.2

A commercial ELISA kit has been evaluated and found to have an overall diagnostic sensitivity of 91% and specificity of 86%, with cross-reactivity being seen with sera from patients with strongyloidiasis, trichinosis, filariasis, and fascioliasis. However, because these infections tend to be infrequent, potential cross-reactivity may not be relevant unless the geographic area includes a high prevalence of any of the above-mentioned infections (61).

Although many cases of VLM are diagnosed by serologic testing, toxocariasis has generally been defined as an infection with *Toxocara* spp., with no attempt to identify the species involved. Studies involving preabsorption of patient sera with cross-reacting antigens and review of follow-up reactivity changes in the sera have confirmed the ability to specifically identify the infecting parasite as *T. canis* or *T. cati*. This ability to distinguish between the two species should be helpful in further biological, epidemiologic, and clinical studies of toxocariasis (62). In cases of encephalitis and myelitis with CSF eosinophilia, parasitic infection should be suspected and appropriate serologic tests should be performed. Other more unusual presentations have included thrombocytosis and eosinophilic pleural effusion. The probability of hepatic toxocariasis can be further evaluated using imaging techniques and ultrasonography. Findings would include focal ill-defined hepatic lesions, hepatosplenomegaly, biliary dilatation, sludge, and periportal lymph node enlargement (63).

In one study, pseudocystic transformation of the peripheral vitreous appeared to be a rather specific and sensitive ultrasonographic biomicroscopic sign in patients with presumed peripheral toxocariasis, and this finding would aid diagnosis in difficult cases (64). However, in a group of 48 patients with diverse inflammatory conditions of the retroiridal space who were examined by ultrasonographic biomicroscopy, no characteristic *Toxocara*-associated pseudocystic images were seen.

To estimate the prevalence of *Toxocara* spp. infection in a representative sample of the U.S. population ≥6 years of age, sera from participants in the Third National Health and Nutrition Examination Survey (1988–1994) were tested for antibodies to *Toxocara*. The age-adjusted *Toxocara* seroprevalence was 13.9%, and it was higher in non-Hispanic blacks (21.2%) than in non-Hispanic whites (12%) or Mexican Americans (10.7%). Seroprevalence was higher for persons 12 to 19, 20 to 29, and 30 to 39 years of age as compared with 6–11 years of age and was higher among males, those living in poverty, individuals born outside of the United States, those living in nonmetropolitan areas, those with above-normal blood lead concentrations, dog owners, and those living in the three geographic regions outside of the West. Seroprevalence was also higher for those persons whose head of household had less than or at least a high school education compared with those with more than a high school education (65).

KEY POINTS—LABORATORY DIAGNOSIS

***Toxocara canis* and *T. cati* (Visceral and Ocular Larva Migrans)**

1. Biopsy specimens are usually not recommended.
2. Serologic tests are recommended. Serum samples can be sent to the appropriate state department of public health (check the applicable state submission requirements). These specimens are often sent to CDC. History information is required, and each sample must be specified as "serum" *or* "eye fluid," so that a correct interpretation of the results can be made. Various reference laboratories also provide the recommended EIA procedure.
3. Tissue specimens containing larvae can be referred to a reference center.

Treatment

Diethylcarbamazine, thiabendazole, ivermectin, mebendazole, and albendazole are effective in some cases but not in others. Corticosteroids may also be given to patients with VLM or OLM. Destruction of the larva by photocoagulation is recommended when the larva is visible in the eye. (Specific drug and dosage information is provided in chapter 36.) Even when the eye is involved, the prognosis is usually favorable, particularly when a prompt diagnosis is made and treatment is effective. Albendazole is the treatment of choice. Although mebendazole is poorly absorbed outside the gastrointestinal tract, it has been used with some success (59).

Epidemiology and Prevention

Given that (i) *Toxocara* worms are commonly found in dogs and cats, (ii) puppies and kittens are infected early in life, and (iii) pets and children are often found in the same household, it is not surprising that the combination of small children playing in contaminated soil and pets passing large numbers of infective eggs leads to VLM and/or OLM. However, it is important to remember that this disease can also occur in adults. The eggs become infective after about 3 weeks and remain viable in the soil for months. Examination of soil from parks and playgrounds in various areas of the world has demonstrated infective *Toxocara* eggs that contribute to the high infection rate seen in dogs. With an estimated 72 million dogs and 82 million cats in the United States, there is potential for widespread environmental contamination with *Toxocara* spp. eggs.

One preventive measure includes worming dogs and cats periodically with mebendazole to keep them free of worms. Another recommendation is preventing children from eating dirt, particularly soil that could be contaminated by neighborhood or family pets. Proper curbing of dogs in the street during defecation has also been recommended. Another approach involves protection of sandboxes in public parks from *Toxocara* egg contamination. The recommendation is to cover the sandboxes with clear vinyl sheets at night and on rainy days. Given the high prevalence of toxocariasis in areas of poor urban and rural hygiene, improved sanitation and access to clean water may also have important roles.

In summary, the following preventive measures should be emphasized: regular deworming of dogs and cats, beginning at 2 weeks of age; removal of cat and dog feces in places adjacent to homes and children's playgrounds; ensuring that children's sandboxes are covered when not being used; regular hand washing after handling soil and before eating; and teaching children not to put dirty objects into their mouths. As a potential explanation for the high rates of asthma and developmental delays among disadvantaged children in poor urban and rural areas, there is an urgent need to fully explore the contribution of toxocariasis to these conditions. Recognition of toxocariasis as a common parasitic disease in the United States and a more significant problem in developing countries is critical for decreasing this neglected infection.

Ancylostoma braziliense and *A. caninum* (Cutaneous Larva Migrans)

Cutaneous larva migrans (CLM), also called creeping eruption, was recognized as a clinical syndrome before the 1800s. Various reports were published during the late 1800s; however, it was not until 1926 that the most common etiologic agent of CLM in the southern United States was found to be *Ancylostoma braziliense*, a very common hookworm of dogs and cats. *A. caninum*, the common hookworm of dogs, has been implicated in cases of CLM. Other species are also capable of producing CLM, although they are less common than *A. braziliense.*

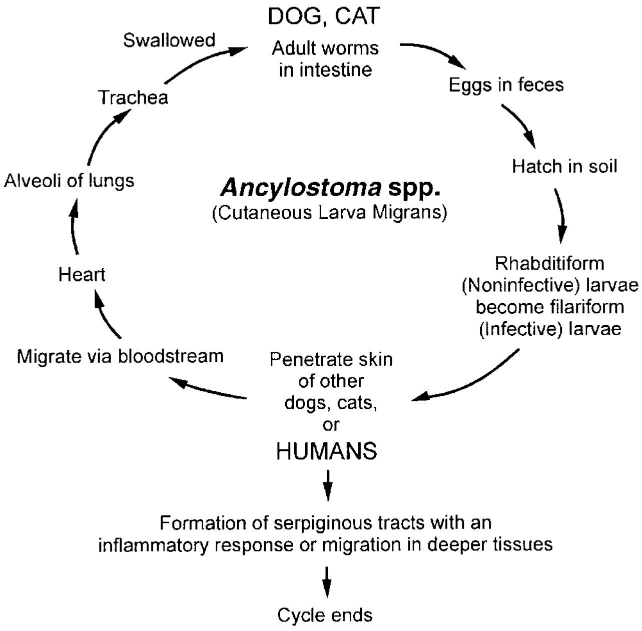
Life Cycle and Morphology

Infection in humans is acquired through skin penetration by infective larvae from the soil (Fig. 14.14). These larvae can also cause infection when ingested. When the larvae penetrate the skin, they produce pruritic papules, which after several days become linear tracks that are elevated and vesicular (Fig. 14.15). Movement by the larvae in the tunnel may extend the track several millimeters each day.

Clinical Disease

Within a few hours after larval penetration of the skin, an itching red papule develops. As the worm begins to migrate from the area of the papule, a serpiginous track appears (Fig. 14.15). The surrounding tissues are edematous and very inflamed. The larva continues to migrate several centimeters each day, and the older portion of the track dries and becomes scarred. This process is associated with severe pruritus, and scratching can lead to secondary infection. Larvae that first enter the skin and cause creeping eruption may later migrate to the deeper tissues (lungs). Deeper-tissue migration may lead to pneumonitis with larval recovery in the sputum (66). A peripheral eosinophilia, as well as many eosinophils and Charcot-Leyden crystals in the sputum, may also be present. In rare cases, CLM may be complicated by Loeffler's syndrome. Apparently, this syndrome results from a type I hypersensitivity reaction related to the pulmonary larval migration phase of various parasites. Clinical findings include migratory pulmonary eosinophilic infiltrates and peripheral eosinophilia, with malaise, fever, and cough.

Figure 14.14 Life cycle of *Ancylostoma* spp., the cause of cutaneous larva migrans (CLM).
doi:10.1128/9781555819002.ch14.f14


DOG, CAT
Adult worms in intestine
Eggs in feces
Hatch in soil
Rhabditiform (Noninfective) larvae become filariform (Infective) larvae
Penetrate skin of other dogs, cats, or
HUMANS
Migrate via bloodstream
Heart
Alveoli of lungs
Trachea
Swallowed
Ancylostoma spp.
(Cutaneous Larva Migrans)
Formation of serpiginous tracts with an inflammatory response or migration in deeper tissues
Cycle ends

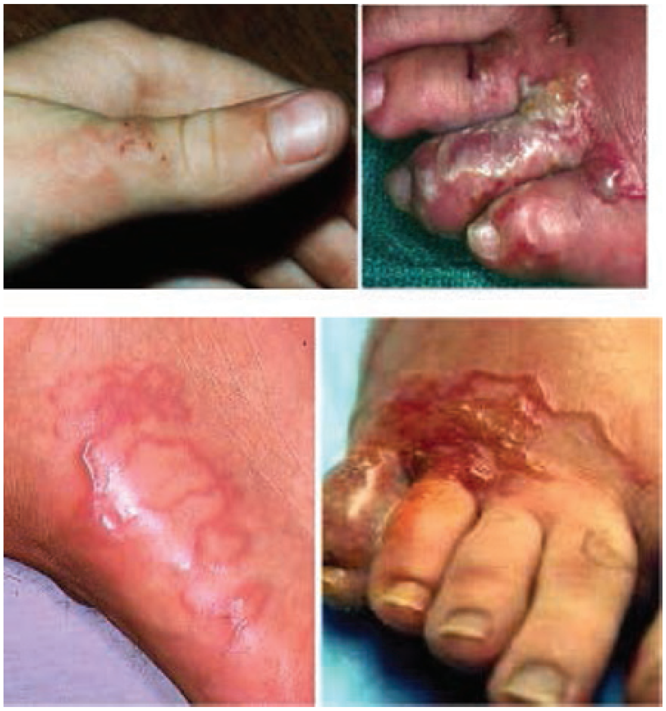


Figure 14.15 Linear tracks caused by migration of *Ancylostoma* spp. [cutaneous larva migrans (CLM)]. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch14.f15

Diagnosis

Diagnosis can usually be made on the basis of the characteristic linear tunnels or tracks and a history of possible exposure; however, other organisms (less common) can also cause CLM. The differential diagnosis would include erythema migrans of Lyme borreliosis, impetigo, scabies, or tinea pedis.

KEY POINTS—LABORATORY DIAGNOSIS

***Ancylostoma braziliense* and *A. caninum* (Cutaneous Larva Migrans)**

1. Diagnosis is usually based on possible exposure history and/or the presence of the linear tracks. Biopsy is not recommended. However, newer PCR methods for the detection and identification of larvae in human tissues may provide improved test results.
2. There may be an elevated eosinophilia (peripheral or sputum).

Treatment

Treatment is generally carried out with thiabendazole, which can be administered either by mouth or topically. Specific drug dosages are provided in chapter 36. Symptoms can also be treated with antihistamines, antipruritic agents, sedatives, and/or topical anesthetics. Typically, the pruritus resolves 24 to 48 hours after treatment, with resolution of the lesions after 1 week.

Epidemiology and Prevention

Most infections are acquired from contact with larvae in moist or sandy soil. Such areas include beaches and sandboxes. Dogs and cats tend to defecate in such areas, providing a perfect situation for accidental infection with the filariform larvae. Specific preventive measures include covering all sandboxes when they are not being used, keeping dogs and cats away from beaches, and periodic deworming of domestic dogs and cats.

Human Eosinophilic Enteritis

As a general definition, primary eosinophilic gastrointestinal disorders selectively affect the gastrointestinal tract, with eosinophil-rich inflammation, in the absence of known causes of eosinophilia, including parasitic infections, drug reactions, and malignancy. Becoming more common, these disorders include eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis (EE), and eosinophilic colitis. It is well known that eosinophils are important members of the gastrointestinal mucosal immune system and that eosinophilic gastrointestinal problems involve mechanisms that include IgE-mediated and delayed Th2-type responses. Based on the site of involvement, primary EE has been classified into three subtypes, namely mucosal, muscular (or mural), and subserosal (or serosal) forms. One or more of these layers can be affected and the symptoms vary accordingly. Predominant mucosal involvement causes diarrhea, subserosal involvement causes ascites, while muscular involvement can lead to thickening and induration of the intestinal wall, resulting in obstruction and abdominal pain. Abnormal eosinophilic infiltration of the intestine is defined by the presence of at least 20 eosinophils per high-power field which can be either diffuse or multifocal. However, peripheral eosinophilia is not always present. Associations have been made with collagen vascular disease, malignancy, food allergy, parasitic or viral infections, inflammatory bowel disease, and drug sensitivity.

Segmental eosinophilic inflammation of the gastrointestinal tract may occur as an isolated condition or as part of a multisystem problem. During the past 20 years, an increasing number of cases have been reported in northern Queensland, Australia (67, 68). All of the patients were Caucasians, and they ranged in age from 16 to 72 years, with no previous illness. They presented with severe abdominal pain, occasional diarrhea, weight loss, and dark stools; all cases were associated with eosinophilia and elevated levels of IgE in serum.

The conclusion that the etiologic agent was *A. caninum* was determined for the following reasons. A single adult *A. caninum* worm was found within a segment of inflamed ileum of one patient. Human hookworms do not occur in urban Australia, and no hookworm eggs were being passed in the stool. In contrast, all of the patients were closely associated with dogs, almost all of which were infected with hookworms. Also, all patients treated with anthelmintic agents responded with a return to normal peripheral blood eosinophil counts. The similarities between this case and the 33 previously reported cases (67) implicate *A. caninum* as the cause of EE in that group of patients. It has been speculated that *A. caninum* causes human EE by inducing allergic responses to its secretions, including cysteine proteinases, which are involved in pathogenesis in other parasites. Immunologic studies involving ELISA and Western blotting for IgG and IgE antibodies to excretory-secretory antigens from adult *A. caninum* also suggest that this parasite is a major cause of EE and peripheral blood eosinophilia (69).

The combination of an affluent, rapidly growing Caucasian population with large numbers of infected dogs as pets, human exposure to infective larvae in a tropical climate with appropriate temperature and humidity, and advanced medical facilities led to the recognition of this association between the parasite infection and subsequent human disease. It also appears that climate directly influences the rate of human enteric infection by canine hookworms (70). Although there are other causes of EE, this disease entity may become more commonly recognized in other areas of the world, thus confirming the causative agent as the common dog hookworm (68) (Table 14.13).

Mebendazole, pyrantel pamoate, and albendazole have been used for the treatment of EE. However, unless there is an awareness of this relatively complex syndrome, a parasitic causative agent may not be considered in the differential diagnosis.

TABLE 14.13 Differential diagnosis for human eosinophilic gastroenteritis[a]

Systemic disorders	Vasculitis: Churg-Strauss syndrome or polyarteritis nodosa Connective tissue disease: scleroderma, dermatomyositis, eosinophilia-myalgia syndrome Others: idiopathic hypereosinophilia, mastocytosis, histocytosis X, nonlipid histiocytosis, eosinophilic granuloma
Tumors	Carcinomas, lymphomas
Intestinal	Inflammatory bowel disorders Intestinal perforation
Food allergies	Cow milk enteropathy
Parasites	Parasites (*Ancylostoma caninum*, giardiasis, strongyloidosis, other zoonoses)
Toxins	Drugs (aspirin, sulfonamides, penicillin, cephalosporin, carbamazepine, azathioprine, L-tryptophan, and gold salts)

[a] Adapted from Oh HE, Chetty R, *J Gastroenterol* **43**:741–750, 2008, and Ekunno N et al, *J Am Board Fam Med* **25**:913–918, 2012.

Dracunculus medinensis

Some people speculate that the "fiery serpent" of biblical times was, in fact, *Dracunculus medinensis* (1). The clinical syndrome was well known in ancient Egypt and during the Greek and Roman periods (1). The contemporary term "guinea worm disease" derives its name from a European explorer who named the disease for the geographic area in which it was found, along the western African coast. The staff of Aesculapius, Roman god of medicine, may have originated from the ancient, still used procedure of removing the adult worm by slowly winding it around a stick (Fig. 14.16). Although the worms are very long and thin, they are not true filarial worms but, rather, are grouped in their own order. Most countries, including Asia, are declared free from the guinea worm disease; thus, the burden of transmission remains in Africa, especially Chad, Ethiopia, Mali, and South Sudan (73).

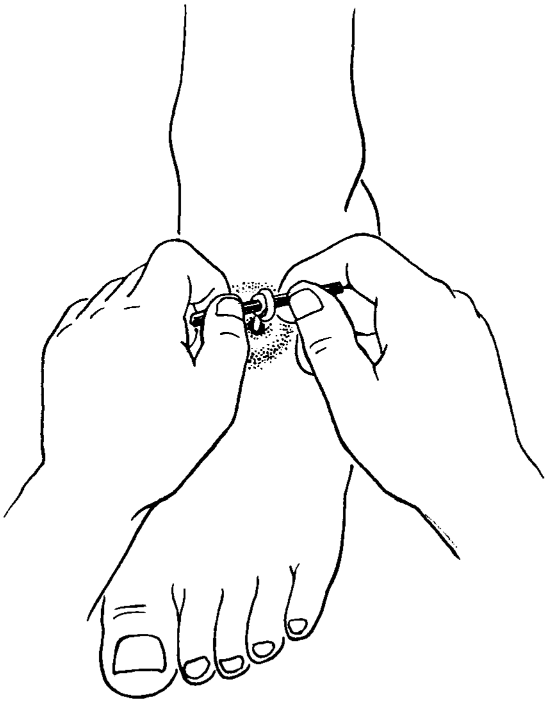
Dracunculiasis was rediscovered in Chad in 2010 after an apparent absence of 10 years; it is unknown whether the infection in Chad was reintroduced in recent years, or had continued at very low levels without detection. The current epidemiologic disease pattern is unlike that seen previously in Chad or other countries of endemicity, including no clustering of cases by village, no association with a common water source, a small number of worms per person, and a large number of infected dogs. Molecular sequencing suggests these infections were all caused by *D. medinensis*. Apparently, the infection in dogs serves as the major factor sustaining transmission in Chad, an aberrant life cycle involving a paratenic host common to people and dogs is occurring (possibly fish, frogs, or other animal as seen in other dracunculid infections), and the cases in humans are sporadic (73).

Life Cycle and Morphology

Human infection is acquired from ingestion of infected copepods (*Cyclops* water fleas) (Fig. 14.17). The released larvae penetrate the duodenal mucosa and develop in the loose connective tissue. The possibility also exists that paratenic hosts, such as tadpoles and frogs, are important means of transporting infective larvae of *Dracunculus* species up the food chain, thus facilitating transmission to the definitive hosts.

The worms are very long, with the females measuring up to 1 m in length by 2 mm in width. The male is much smaller and inconspicuous (2 cm long). The worms mature in the deep connective tissue, and the females migrate to the subcutaneous tissues when they are gravid and contain coiled uteri filled with rhabditiform larvae. Maturation takes approximately 1 year. At this stage in the life cycle, the female migrates to the skin and a papule is formed in the dermis, usually by the ankles or feet (although papules can be anywhere on the body). The papule changes into a blister within 24 h to several days. Eventually, the blister ulcerates, and on contact with freshwater, a portion of the uterus prolapses through the worm's body wall, bursts open, and discharges thousands of larvae into the water (Fig. 14.18 and 14.19). This may happen several times

Figure 14.16 *Dracunculus medinensis*, blister on leg (contains female worm); note removal of the adult worm. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch14.f16



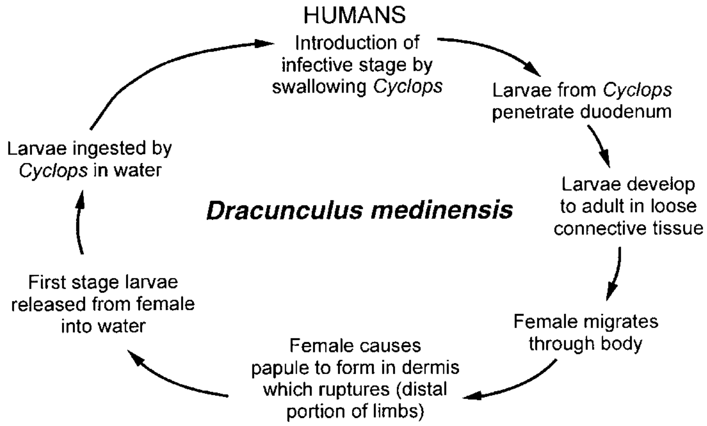

HUMANS
Introduction of infective stage by swallowing *Cyclops*
Larvae from *Cyclops* penetrate duodenum
Larvae develop to adult in loose connective tissue
Female migrates through body
Female causes papule to form in dermis which ruptures (distal portion of limbs)
First stage larvae released from female into water
Larvae ingested by *Cyclops* in water
Dracunculus medinensis

Figure 14.17 Life cycle of *Dracunculus medinensis*, the cause of guinea worm disease. doi:10.1128/9781555819002.ch14.f17

until all of the larvae are discharged. The larvae are then ingested by an appropriate species of *Cyclops*. Development takes about 8 days before the larvae are infective for humans.

Although the adult worms are often described as creamy white, there are reports of red worms that appear to be female *D. medinensis*. These infections occurred in an area of Pakistan where the incidence of guinea worm in 1988 was 15%. Unfortunately, examination of histologic sections was unable to determine the cause of the red color; however, blood was excluded as a possible cause.

Clinical Disease

After ingestion of an infected copepod, no specific pathologic changes are associated with larval penetration into the deep connective tissues and maturation of the worms. Once the gravid female begins to migrate to the skin, there may be some erythema and tenderness in the area where the blister will form. Several hours before blister formation, the patient may exhibit some systemic reactions, including an urticarial rash, intense pruritus, nausea, vomiting, diarrhea, or asthmatic attacks. The lesion develops as a reddish papule, measuring 2 to 7 cm in diameter. Symptoms usually subside when the lesion ruptures, discharging both the larvae and worm metabolites.

In a 1995 study in Nigeria, 1,200 people were surveyed for dracunculiasis. Many (982 [82%] of 1,200) were infected, and most infections involved the lower limbs (98%). Worms were also seen emerging from the umbilicus, groin, palm, wrist, and upper arm. Of the 982 infected individuals, 206 (21%) were totally incapacitated, 193 (20%) were seriously disabled, 431 (44%) were mildly incapacitated, and 152 (16%) were unaffected (74).

If the worms are removed at this time, healing usually occurs with no problems. If the worm is damaged or broken during removal, there may be an intense inflammatory reaction with possible cellulitis along the worm's migratory track. If secondary infection occurs, there may be serious sequelae, including arthritis, synovitis, and other symptoms, depending on the site of the lesion.

Diagnosis

Diagnosis can be confirmed at the time the cutaneous lesion forms, with subsequent appearance of the adult worm. Infected lesions must be distinguished from carbuncles, deep cellulitis, focal myositis or periostitis, and even rheumatism. Calcified worms may also be found in subcutaneous tissues by radiography. They may appear as linear densities (up to 25 cm), tightly coiled structures, or sometimes nodules. Depending on the site, they can also be misdiagnosed as possible breast cancer.

Treatment

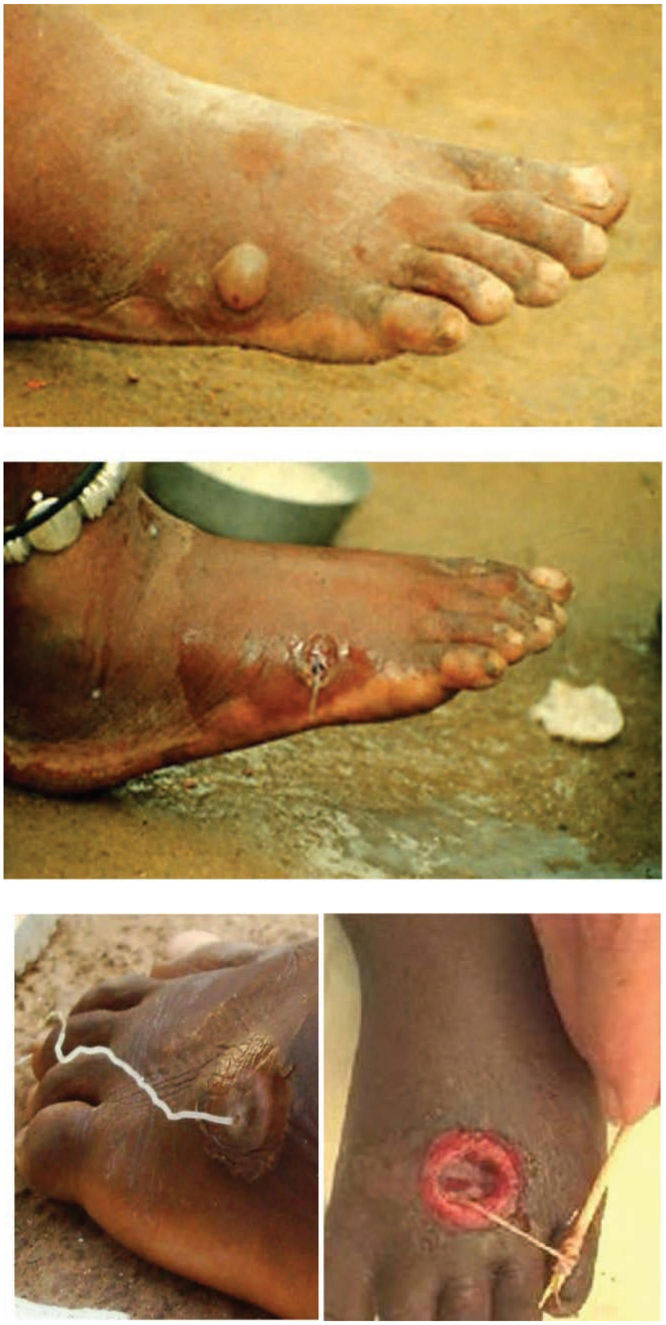
For centuries, the worms have been removed by slowly being wound around a stick (Fig. 14.18). This approach works well unless the worm is accidentally broken and secondary infection occurs. Allergic manifestations can be decreased by using epinephrine (1).

Four drugs have been used with various degrees of success: niridazole, thiabendazole, metronidazole, and mebendazole. The action seems to involve suppression of inflammation rather than any specific effect on the adult worms, although 400 to 500 mg/day for 6 days has been reported to kill the worms directly (41). The prognosis is usually quite good unless there are complications such as chronic recurrent nodules and ulcers, aberrant migration of the worms, or calcification of the adult worms. Specific drug dosage information is provided in chapter 36.

Epidemiology and Prevention

Disease transmission depends on several factors: (i) water sources where *Cyclops* spp. breed, (ii) direct contact between infected humans and the water source, (iii) use of this water source for drinking, or (iv) the possibility of other paratenic hosts. In various parts of the world, certain types of water sources (e.g., step wells in India, covered cisterns in Iran, and ponds in Ghana) provide all of these transmission requirements. The disease can be eliminated within 1 to 2 years by provision of safe drinking water. However, with the current unusual epidemiologic pattern in Chad, other control possibilities may have to be taken into account (73, 75).

Figure 14.18 *Dracunculus medinensis*, progression from blister through worm removal. (Upper two images courtesy of the CDC Public Health Image Library; lower two images from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman, photograph courtesy of J. Donges.) doi:10.1128/9781555819002.ch14.f18



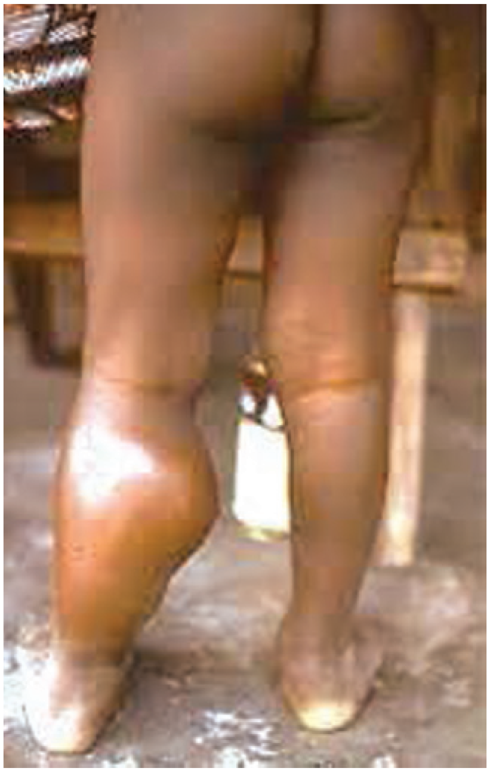


Figure 14.19 *Dracunculus medinensis*, abscess caused by developing worm. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch14.f19

In 1986, approximately 3.5 million cases of dracunculiasis occurred in 20 countries, and 120 million people were at risk for the disease. The target date for eradication of guinea worm infection of 1995 had been set by the African Regional Office of the World Health Organization and accepted by the United Nations Children's Fund and the United Nations Development Program. Although much progress has been achieved, there remain some obstacles to eradication (76). Through January of 2013 Asia was free from dracunculiasis, and no cases were reported worldwide for the first time since the eradication program began in 1986 (75). It is hoped that this infection will eventually join smallpox as one that can actually be eradicated from the world.

Angiostrongylus (*Parastrongylus*) *cantonensis* (Cerebral Angiostrongyliasis)

Human infection with the rat lungworm *Angiostrongylus cantonensis* was first detected in 1945 in a 15-year-old Taiwanese boy with suspected meningitis. It has been recognized in the Pacific areas for many years, with Thailand, Tahiti, and Taiwan being areas of high endemicity. Sporadic cases have also been reported in other parts of the world, including Australia, Fiji, Sri Lanka, Egypt, Madagascar, Central America, Jamaica, and Cuba. The first case in the United States was reported in 1995 in a patient from New Orleans (77). Apparently, many gastropods in New Orleans are competent hosts for *A. cantonensis*. Also, the presence of infected rats and primates (at the Audubon Zoo) indicates that there is a reservoir of infection in New Orleans. The infection is associated with eosinophilic meningitis and sometimes eye involvement (78). Currently there are four species within the genus: *A. cantonensis*, *A. costaricensis*, *A. malaysiensis*, and *A. mackerrase* (these four are now placed in the new genus *Parastrongylus*). Since most parasitologists may continue to use the genus name *Angiostrongylus*, this generic designation is used throughout this chapter.

Life Cycle and Morphology

Mature adult worms of *A. cantonensis* inhabit the pulmonary arteries of a wide variety of rodents, primarily those within the genera *Rattus* and *Bandicota* (Fig. 14.20). Eggs laid by the female lodge in the pulmonary arteries. On hatching, the first-stage larvae enter the alveolar space, migrate up the trachea and down the alimentary tract, and are excreted in the feces. Terrestrial snails, slugs, and aquatic snails serve as intermediate hosts, either by first-stage larval penetration of tissues or by the ingestion of contaminated rodent feces. Larval development continues in the mollusk, where third-stage, infective larvae develop within about 2 weeks. When ingested by rodents, the infective third-stage larvae migrate to the brain via the circulation and develop into fourth-stage larvae and then young adults within 4 weeks. They then go to the subarachnoid space, enter the venous system, and arrive in the pulmonary arteries, where sexual maturity occurs within another 2 weeks.

Human infection begins with the accidental ingestion of infective larvae in several species of slugs, snails, or land planarians (Fig. 14.21). Ingestion of infected raw paratenic hosts, including fish, amphibians, reptiles, crustaceans, and vegetables contaminated with larvae, also leads to infection of the human host. Survival of the fifth-stage larvae is not certain; some probably die in the brain and spinal cord while some reach the eye chamber; very few probably reach the lungs. In the natural life cycle, infective larvae have also been found in land crabs, coconut crabs, and freshwater prawns, which are often consumed raw in the Pacific Islands. In summary, human infection can originate through the following: by ingestion of L3 larvae in raw or undercooked intermediate or paratenic hosts, by drinking infected water, by oral contact with hands contaminated with mollusk larvae, or possibly through the skin.

The worms are very thin and delicate, measuring 17 to 25 mm long by 0.26 to 0.36 mm wide. The young adults within the brain tissue are approximately 2 mm long.

Clinical Disease

The incubation period is normally around 20 days but may be twice that. The main symptom in all reported

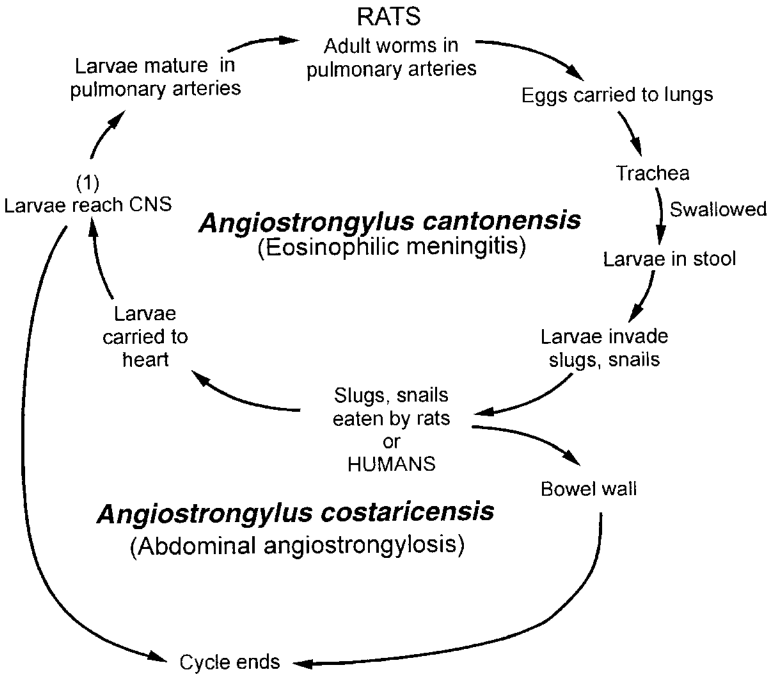

RATS
Adult worms in pulmonary arteries
Eggs carried to lungs
Trachea
Swallowed
Larvae in stool
Larvae invade slugs, snails
Slugs, snails eaten by rats or HUMANS
Bowel wall
Cycle ends
Larvae carried to heart
(1) Larvae reach CNS
Larvae mature in pulmonary arteries
Angiostrongylus cantonensis
(Eosinophilic meningitis)
Angiostrongylus costaricensis
(Abdominal angiostrongylosis)

Figure 14.20 Life cycle of *Angiostrongylus* (*Parastrongylus*) spp. [see (1) in figure]. Humans present with encephalitis when worms die in the brain tissue. doi:10.1128/9781555819002.ch14.f20

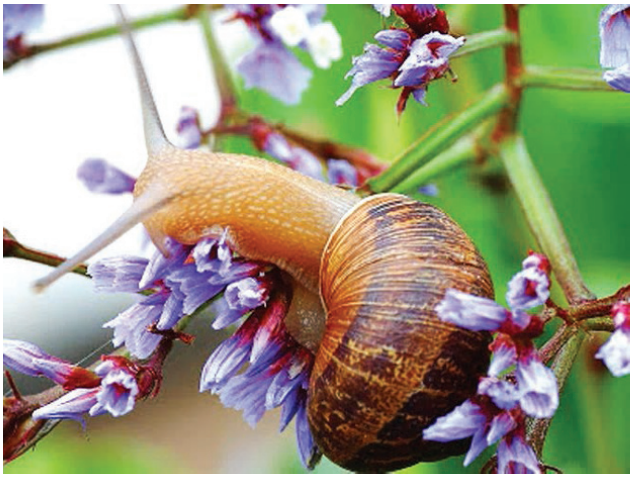


Figure 14.21 *Angiostrongylus* (*Parastrongylus*) *cantonensis*. Snail vector (common garden snail). (Courtesy of Majik Phil blog, www.majikphil.blogspot.com.)
doi:10.1128/9781555819002.ch14.f21

cases of meningitic angiostrongyliasis was severe headache. Other symptoms include convulsions, weakness of the limbs, paresthesia, vomiting, constipation, nausea, anorexia, facial paralysis, neck stiffness, and fever. Pulmonary symptoms are usually absent, although immature adult worms have been seen in lung tissue sections. Recovery of larvae in sputum or stool has not been reported. The spinal fluid usually contains white blood cells (100 to 2,000/mm^3 with many eosinophils). There is often a peripheral eosinophilia with moderate leukocytosis. In most cases, the disease is self-limiting and the patient recovers within a month. There are three major forms of human angiostrongyliasis: meningitic, encephalitic, and ocular; the majority of patients exhibit the meningitic form (Fig. 14.22).

Eye involvement is characterized by visual impairment, pain, possible retinal hemorrhage, and retinal detachment. Living worms have been removed in some cases (Fig. 14.23). Ocular angiostrongyliasis has been reported from Thailand, Taiwan, Vietnam, Indonesia, Japan, Papua New Guinea, and Sri Lanka. Worms have been found in the anterior chamber, retina, and other sites, causing multiple symptoms and rare cases of blindness.

A documented patient from New Orleans was an 11-year-old boy who presented with myalgia, headache, low-grade fever, and vomiting. He had no travel history but admitted that, on a dare, he had eaten a raw snail from the street a few weeks earlier. While serologic tests for VLM and *Toxoplasma gondii* were negative, the results for *A. cantonensis* were positive (77).

Most patients, including the one from New Orleans, recover uneventfully and do not require hospitalization. Symptoms gradually disappear, with the meningeal prob-

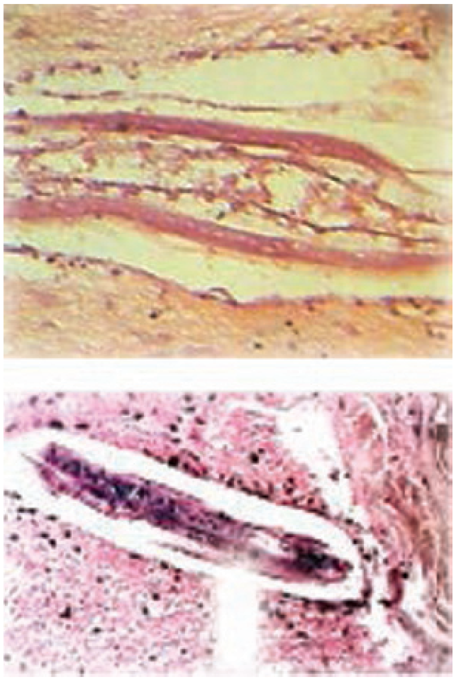


Figure 14.22 *Angiostrongylus* (*Parastrongylus*) *cantonensis*. (Upper) Longitudinal parasite within the meninges (courtesy of Alejandro Ruiz Mendoza, VI Congreso Vitual Hispanoamericano de Anatomía Patológica, 2004). (Lower) Larva penetrating the arachnoid (courtesy of picasaweb.google.com).
doi:10.1128/9781555819002.ch14.f22

Figure 14.23 *Angiostrongylus* (*Parastrongylus*) *cantonensis* adult male worm in the eye. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman; original image by John Cross.)
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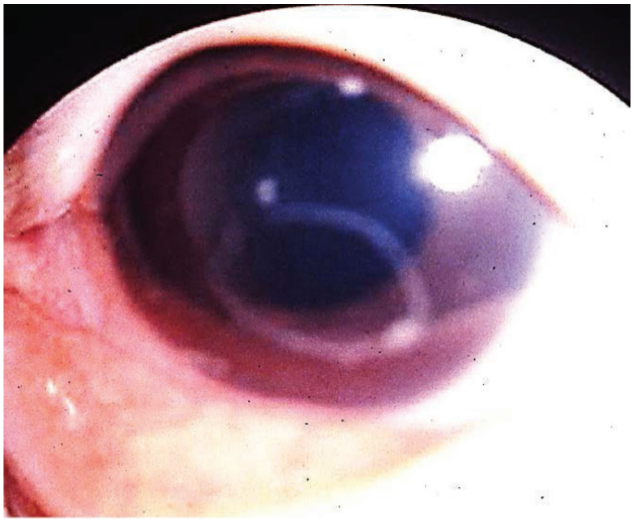


lems resolving first, followed by the visual abnormalities and finally by the paresthesia. Although rare, the infection can be fatal.

Diagnosis

Definitive diagnosis of infection with *A. cantonensis* would require identification of larvae or young adults in human tissue, such as the brain, CSF, and eye chamber, all of which can be difficult. Thus, the diagnosis is usually made on the basis of serologic test results. A presumptive diagnosis can be made in areas where infections are endemic on the basis of symptoms of severe headache, meningitis, or meningoencephalitis, with fever and ocular involvement. A peripheral eosinophilia and eosinophils in the CSF would also be highly suggestive. Lesions can also be seen in the brain by computed tomography. Larvae or young adult worms can often be recovered in the CSF, and the serologic ELISA can also provide confirmation. Both adult and young *A. cantonensis* worm antigens purified by immunoaffinity chromatography have been used to detect antibody in serum and CSF by ELISA. Infected patients had increased levels of IgG, IgA, IgM, and IgE, with higher IgM and IgE levels in serum than in CSF. Both worm antigens were highly sensitive in ELISA for serum antibodies but less so in tests for antibodies in CSF (79). More advanced molecular methods are under development.

Treatment

If the worm is found in the eye, surgical removal of the worm is normally recommended. Anthelmintic agents are usually not used, although both mebendazole and thiabendazole have been tried. Mebendazole is currently the drug of choice (41). These drug trials have been inconclusive, and symptomatic therapy is normally recommended. However, since pathogenesis is frequently ascribed to dead or dying worms, anthelmintics should be used with caution. Corticosteroids may also eliminate some symptoms.

Epidemiology and Prevention

The lack of host specificity, the natural mobility of rats, and the expansion of the geographic range of the large African land snail have all contributed to the spread of this infection throughout the tropical and subtropical areas of the world. It is often difficult to identify the specific source of human infections; however, awareness of the various possible hosts may decrease the number of infections. Angiostrongyliasis is an emerging public health problem in mainland China (80). No overall control measures have been recommended. However, control of the spread of infected rats and mollusks to areas where *A. cantonensis* is not endemic will help restrict the geographic range.

Angiostrongylus (*Parastrongylus*) *costaricensis* (Abdominal Angiostrongyliasis)

At the time *Angiostrongylus costaricensis* was described in 1971, the natural host was unknown. The cotton rat, the black rat, and a number of other rodents harbor the adult worms, while various slugs harbor the larvae. Human infections are most common in Costa Rica (where there are about 600 per year) but have been reported in other areas of Mexico and Central and South America.

Life Cycle and Morphology

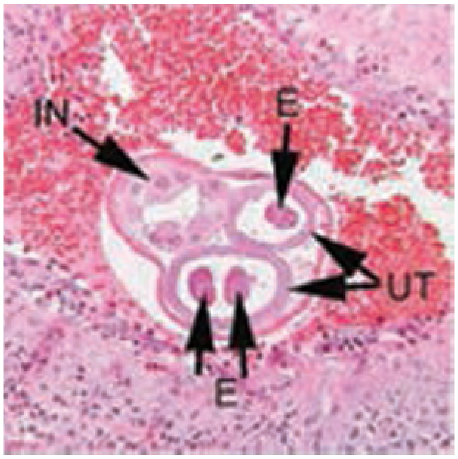
The life cycle is similar to that of *A. cantonensis*, with human infection being initiated by accidental ingestion of the appropriate slug, frequently on contaminated salad vegetables (Fig. 14.21). This infection is called abdominal angiostrongyliasis, and the worms cause inflammatory lesions of the bowel wall.

The eggs are oval and about 90 μm long, have a thin shell, and are unembryonated (they may be embryonated in humans, with some larvae being released from the egg). The adult worms measure 42 by 350 mm (females) and 22 by 140 mm (males).

Clinical Disease

Abdominal angiostrongyliasis in found mainly in children under 13 years, and some groups have reported that two-thirds of these are male. The appendix is often involved; however, the worms can also be found in the terminal ileum, cecum, ascending colon, regional lymph nodes, and mesenteric arteries (Fig. 14.24). There may be inflammation, thrombosis, and regional necrosis, with granulomas and areas of eosinophilic infiltrates around eggs and larvae

Figure 14.24 *Angiostrongylus* (*Parastrongylus*) *costaricensis.* Female worm in appendix (hematoxylin and eosin stain). IN, multinucleate intestine; E, eggs; UT, uterus. (Courtesy of Regions Hospital, St. Paul, MN, and the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch14.f24



in various stages of development. The most common symptoms are pain and tenderness, with a palpable mass in the lower right quadrant, along with fever and possibly vomiting and diarrhea. Occasionally, the worms are present in the liver; the symptoms may mimic those of VLM. Leukocytosis is present, with eosinophilia of up to 80%. Clinical symptoms occur about 2 weeks after infection and include abdominal pain in the right iliac fossa and right flank, fever, anorexia, vomiting, diarrhea, and constipation.

Diagnosis

Eggs or larvae may be seen in tissue sections, with most specimens being diagnosed on microscopic findings. Without histologic sections, the diagnosis is made on clinical grounds. Radiology may reveal abnormalities in the terminal ileum, cecum, and ascending colon. Contrast medium studies show spasticity, filling defects, and irritability at the cecum and ascending colon. When the liver is involved, there may be leukocytosis and eosinophilia, as well as elevated liver enzyme levels.

Treatment

Several drugs have been tried; the drug of choice is thiabendazole, with another option being mebendazole (41). Depending on the clinical findings, surgical removal may be another option.

Epidemiology and Prevention

The ingestion of raw slugs in the areas of endemicity is considered strictly accidental. Most of the infections reported from Costa Rica have been in children, with a higher incidence in boys than in girls. Prevention involves rodent control to break the normal parasite cycle, as well as thorough washing of vegetables and other foods prior to consumption.

Recently, *A. costaricensis* has been reported in several primates at the Miami MetroZoo (siamang) and the Monkey Jungle in Miami (Ma's night monkeys). Also *A. costaricensis* was found in an opossum trapped in the MetroZoo, as well as in four raccoons near the MetroZoo. These are the first records from all four species of hosts. The primates were zoo born and the raccoons and opossum were native, thus indicating that this parasite is now endemic at these two sites (81).

Gnathostoma spinigerum

Although many *Gnathostoma* species have been mentioned in the literature, *G. spinigerum* is considered to be the most medically important. This parasite is normally found in dogs and cats; the largest number of infections, both in humans and in reservoir hosts, is found in Thailand (1). Areas of endemicity include China, the Philippines, and other areas in the Far East. Also, the prevalence of such infection is increasing in Mexico and Central and South America. The first record of a confirmed case of *G. doloresi* infection has been reported from Japan; the parasite was dissected from the skin and was identified as a third-stage larva. Although the entire life cycle is not fully understood, the patient in Japan reported eating raw brook trout about 2 months before the onset of the creeping eruption (82). Another case report from Japan describes colonic ileus due to nodular lesions caused by *G. doloresi* (83).

Life Cycle and Morphology

Within the definitive host (cats, dogs, and some wild carnivores), the adult worm lies coiled in a tumor-like mass in the stomach wall. Eggs are extruded from the stomach lesions and are passed out with the feces. These eggs hatch 10 to 12 days after reaching water, releasing first-stage larvae. The larvae are then ingested by copepods, where they develop into second-stage larvae within about 2 weeks. When an infected copepod is then ingested by any of the many intermediate hosts (fish, amphibians, reptiles, birds, and mammals), the third-stage larvae encyst. Once the intermediate host is ingested by the definitive host, the parasites become localized in the stomach wall, where they mature in 2 to 12 months (84).

Human infections are acquired by the ingestion of raw or poorly cooked or pickled freshwater fish, chicken and other birds, frogs, or snakes (Fig. 14.25). There is also speculation that human infection can occur from the ingestion of copepods containing the advanced third-stage larvae or by actual skin penetration of larvae from handling infected meat. Three cases of parasitologically proven gnathostomiasis in neonates have been reported; these infections were presumably transmitted prenatally or perinatally from mother to infant (84). In humans, the ingested third-phase larvae do not mature but initiate a dramatic tissue migration phase. The destructive nature of gnathostomiasis occurs through direct mechanical- and toxin-mediated injury, as well as from local host inflammatory reactions to the migrating larvae.

The adult worms, which are found in the stomach lesions, are 25 to 54 mm (females) and 11 to 25 mm (males) long. The anterior half of the worm is covered with leaflike spines (Fig. 14.26 and 14.27).

Clinical Disease

In most cases, the incubation period is difficult to determine. The disease is usually seen in two very different clinical forms, larval gnathostomiasis and eosinophilic myeloencephalitis. Symptoms can recur over a period of 10 to 12 years.

Larval Gnathostomiasis. The cutaneous manifestations of *Gnathostoma* infection include panniculitis, creeping eruptions, and pseudofurunculosis with a predilection to the

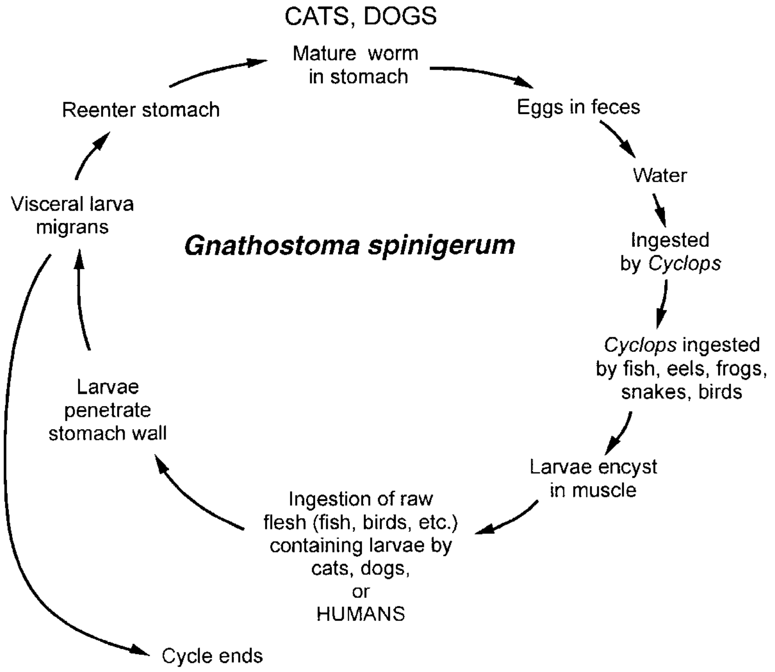
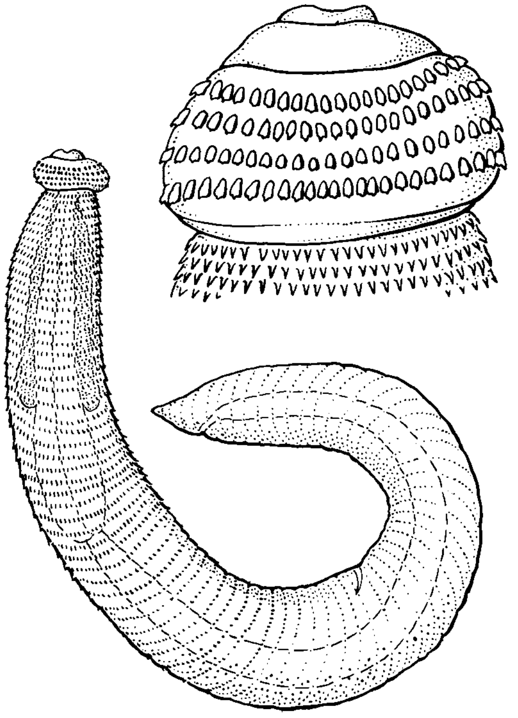

CATS, DOGS
Mature worm in stomach
Eggs in feces
Water
Ingested by *Cyclops*
Cyclops ingested by fish, eels, frogs, snakes, birds
Larvae encyst in muscle
Ingestion of raw flesh (fish, birds, etc.) containing larvae by cats, dogs, or HUMANS
Larvae penetrate stomach wall
Visceral larva migrans
Reenter stomach
Cycle ends
Gnathostoma spinigerum

Figure 14.25 Life cycle of *Gnathostoma spinigerum.* doi:10.1128/9781555819002.ch14.f25

Figure 14.26 *Gnathostoma spinigerum*, adult worm. (Illustration by Sharon Belkin, adapted from reference 1; originally adapted from Miyazaki I, *Kyushu Mem Med Sci* 5:123–140, 1954.) doi:10.1128/9781555819002.ch14.f26



trunk. These lesions are associated with recurring pain, pruritis, and erythema. Also, visceral forms are seen, with the visceral larval migration including pulmonary, gastrointestinal, urogenital, ocular, otorhinolaryngeal, and cerebral tissues. In the human host, the larvae do not mature into adults in the wall of the stomach but, rather, migrate throughout the body. Several days after ingestion of the larvae, penetration of the intestinal wall may lead to epigastric pain, vomiting, and anorexia. These symptoms subside as the larvae begin to migrate through the tissues in deep cutaneous or subcutaneous tunnels. Evidence of this migration appears either as lesions similar to those found in patients with CLM or, more commonly, as migratory swellings with inflammation, redness, and pain. This swelling is hard and nonpitting, and may last for several weeks. After it disappears, it may reappear in a location not far from the first swelling, which can be on the upper extremity, shoulder, neck, thorax, face, scalp, abdominal wall, thigh, or foot. Sometimes the lesions are painless, and sometimes there is pain and pruritus. These swellings probably result from the allergic response of the host to the presence of the worms, and an eosinophilia of 35 to 80% is reported in patients with cutaneous involvement. Visceral migration can lead to serious sequelae, depending on the organ(s) affected (Table 14.3). Similar to infections with *Angiostrongylus*, *Gnathostoma* can directly invade the eye and cause pain, uveitis, increased intraocular pressure, and blindness (85).

Eosinophilic Gnathostomiasis. Neurologic manifestations are seen as a complex group of symptoms called eosino-

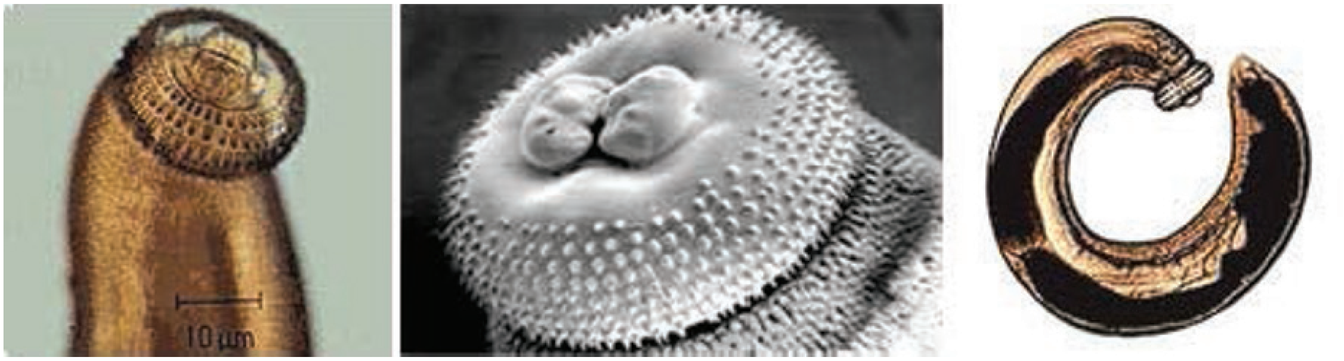


Figure 14.27 *Gnathostoma spinigerum*. (Left to right) Third-stage larva, head; scanning electron micrograph; whole third-stage larva (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch14.f27

philic myeloencephalitis. More serious symptoms can occur if the eyes or CNS is involved. The larval migration occurs along a peripheral nerve, into the spinal cord, and then into the brain. Neurotropic infection is usually severe and presents with radicular pain and paresthesias of the trunk and extremities and, less frequently, with paresis or paralysis. Symptoms include pain, paralysis, seizures, coma, and death. Sudden severe headache and sensory impairment followed by coma can occur and mimic a cerebrovascular accident. In rare instances, cutaneous migratory swellings and neurologic symptoms occur in the same patient. In areas of endemic infection, cerebral hemorrhage in a younger individual should indicate possible infection with *Gnathostoma* spp. The spinal fluid may be xanthochromic or bloody. Ocular invasion probably occurs via the optic nerve, although penetration through the sclera may be possible.

Diagnosis

Although the diagnosis of this infection can be difficult, peripheral and CSF eosinophilia (rare cases seen with no peripheral eosinophilia), history of travel to regions with high risk of infection, and progression of symptoms are highly suggestive (Table 14.14). Patient symptoms may also suggest sparganosis, cutaneous paragonimiasis, CLM, and myiasis. CSF specimens from patients with gnathostomiasis usually show xanthochromia, an elevated opening pressure in one-half of infected patients, pleocytosis with eosinophilia, normal glucose levels, and normal or elevated protein levels. However, CSF that is bloody or xanthochromic and contains many eosinophils also may be suggestive of infection with *A. cantonensis* (86). A definitive diagnosis depends on recovery and identification of the worms. Newer serologic methods may also be helpful; however, some assays are limited by cross-reactivity to other nematodes. Currently, there is no immunologic test for *Gnathastoma* from an accredited (CLIA) laboratory. Biopsies are recommended (personal communication from Irving G. Kagan, 2012).

Treatment

The only effective therapy is surgical incision of the lesion and removal of the worm. Albendazole has also been recommended as an adjunct to surgical removal (41). Worm removal from the eye may also prevent CNS invasion. The prognosis is usually good unless the CNS is involved. Additional therapeutic information is presented in chapter 36.

Epidemiology and Prevention

Most infections are probably caused by the ingestion of raw or poorly cooked fish, domestic ducks, and chickens. The larvae do not survive cooking, and they can be killed by immersion in strong vinegar for at least 5 h. Unfortunately, soaking in lime juice or storage at 4°C for 1 month does not kill the larvae. Untreated groundwater is also a potential problem and source of transmission because it can contain infected copepods. Once the life cycle is established in an area, potential infection can become a serious public health issue for residents. Increases in world travel and the importation of food require heightened awareness of this infection, particularly in areas such as Europe and North America, where the infection is not endemic.

Gnathostoma doloresi, *G. nipponicum*, *G. hispidum*, and *G. binucleatum*

Other *Gnathostoma* species causing human infection include *G. doloresi*, *G. nipponicum*, *G. hispidum*, and *G. binucleatum*. The general life cycle is identical to that of *G. spinigerum*, with slight variations related to the second, paratenic, and definitive hosts. Patient symptoms are also similar to those seen with *G. spinigerum* infections. In Japan, freshwater fish are the most likely source of *G. doloresi*, *G. hispidum*, and *G. nipponicum* (87). In Myanmar, an outbreak among Korean emigrants was reported; 38 of 60 individuals became symptomatic after ingesting raw freshwater catfish, freshwater bream, and

TABLE 14.14 Comparative features of *Gnathostoma* and *Angiostrongylus* infections[a]

Characteristic	*Gnathostoma spinigerum*	*Angiostrongylus cantonensis*	Comments
Infection route	Ingestion of infected raw, poorly cooked, or pickled freshwater fish, chicken and other birds, frogs, or snakes; possible copepod ingestion or skin penetration	Ingestion of raw slugs, snails, land planarians, paratenic hosts (fish, amphibians, reptiles, crustaceans, vegetables contaminated with larvae); infected drinking water, hands contaminated with larvae; through the skin; contact with rats Pacific Islands: land crabs, coconut crabs, freshwater prawns	Very detailed information on travel destinations and food consumption may be extremely helpful.
Geographic distribution	Thailand, China, Philippines, Japan, other areas in Far East; increasing in Mexico, Central/South America	Pacific, Australia, Africa, the Caribbean, Hawaii, Louisiana, Fiji, Sri Lanka, Egypt, Madagascar, Central America, Jamaica, Cuba	A detailed travel history is always recommended, as well as detailed information on food ingestion; people tend to try new dishes when traveling; all information may be relative.
Onset of symptoms	Days to months; difficult to determine	Days to about a month	Not definitive
Duration of symptoms	10 to 12–13 years	Few months	Not definitive
Symptoms	**Cutaneous:** panniculitis, creeping eruptions, and pseudofurunculosis **Visceral:** associated with myiasis. Other pulmonary, gastrointestinal, urogenital, ocular, otorhinolaryngeal, and cerebral tissues **CNS:** radicular pain and paresthesias of the trunk and extremities, paresis or paralysis, seizures, coma, death	**Meningitic disease:** headaches, photophobia, stiff neck, vomiting, anorexia, parasthesias/hyperesthesias, fever, cranial nerve 8 palsy **Ocular involvement:** visual impairment, pain, possible retinal hemorrhage, retinal detachment	Infections with *Gnathostoma* spp. may suggest sparganosis, cutaneous paragonimiasis, CLM, myiasis. Other infections, including *Baylisascaris* infection, toxocariasis, and neurocysticercosis; malignancies; medications; and the presence of intracranial foreign bodies can produce eosinophils in the CSF.
Laboratory findings	**CSF:** xanthochromia, elevated pressure in half of patients, pleocytosis with eosinophilia, normal glucose, normal/elevated protein	Peripheral/CSF eosinophilia **CSF:** elevated open pressure, elevated WBC count, elevated protein, normal glucose	There are many similarities between the two infections. Eosinophils can be distorted or destroyed during CSF processing and can be mistaken for neutrophils if using automation; eosinophils exhibit better morphology with Wright or Giemsa staining.
Imaging findings	**Head CT, MRI:** hemorrhage and hydrocephalus	Head CT, MRI usually normal	Angiostrongyliasis does not usually produce focal lesions on head CT or MRI.
Serology for antibody	Immunoblot; **no routine tests available**	Immunoblot; ELISA test of choice, available in some reference labs	If there is a clinical index of suspicion for angiostrongyliasis, it is important to obtain serological data on convalescent-phase samples; time for seroconversion may be 60+ days.
Treatment	**Cutaneous:** albendazole, ivermectin **CNS:** supportive therapy (steroids) **Overall:** no recommendation for anthelmintics	If eye, surgical removal; mebendazole, thiabendazole tried; symptomatic therapy usually recommended (consider steroids); no general recommendation for anthelmintics (if used, mebendazole or albendazole); symptomatic treatment includes serial lumbar punctures to alleviate increased intracranial pressure and analgesics.	Documented potential benefits of treating angiostrongyliasis with anthelmintics and steroids exist; however, such studies do not exist for neurologic gnathostomiasis. General use of anthelmintics is not common due to concern over inflammatory reactions to dying parasites.

[a] Adapted from references 77–80, 84–86.

snake-headed fish in a local Korean restaurant (88). Although positive serologic test results were based on the use of *G. doloresi* antigen, *G. spinigerum* was suspected as the causative agent and is known to be present in the Yangon area of Myanmar.

An increasing number of human cases have also been reported in Sinaloa, Mexico, most of which occurred in persons who had eaten raw fish dishes such as ceviche. A report confirming five cases from Mexico described the first known outbreak of acute gnathostomiasis on the American continent (89). These patients were seropositive to *G. doloresi* antigen. Five species of fish and four species of ichthyophagous birds collected from three lakes and a nearby estuary were infected with third-stage larvae of *G. binucleatum*, a species found in Ecuador and Mexico.

Anisakis simplex, *A. physeteris*, *Pseudoterranova decipiens*, *Contracaecum osculatum*, *Hysterothylacium aduncum*, and *Porrocaecum reticulatum* (Larval Nematodes Acquired from Saltwater Fish)

Anisakiasis was first recognized and reported in the Netherlands. Since this infection was reported in Japan in 1965, hundreds of Japanese cases have been documented, as have several in the United States (90). Up to 1990, more than 12,000 cases were reported from Japan and only 519 cases were reported in 19 countries outside of Japan, including Spain, France, the Netherlands, and Germany. Approximately 3,000 individuals suffer from *Anisakis* infection each year in Japan. The majority of these cases involve gastric anisakiasis, and colonic anisakiasis is extremely rare. Fewer than 100 cases have been reported from the United States; however, this infection is probably misdiagnosed and underreported. During the past 2 decades, there has been increased documentation of cases of infection from New Zealand, Canada, Brazil, Chile, and Egypt. With the tremendous increase in the popularity of sushi and sashimi, it is likely that the number of case reports will continue to increase over the next few years. It is well recognized that human infection can occur from the ingestion of raw or poorly cooked marine fish or squid. In one fish market in Japan, 98% of the mackerel and 94% of the cod were infected. Pseudoterranovosis rarely occurs in Japan and Europe. However, it occurs more frequently in the United States and Canada, where *P. decipiens* is mainly transmitted by the Atlantic or Pacific cod, Pacific halibut, and red snapper.

In the United States, one study reported a 1 in 13 chance of consuming an anisakid larva in salmon sushi (albeit all were dead from freezing) (90). Other than sushi, high-risk dishes include salted and smoked herring in the Netherlands, Scandinavian gravlax, Hawaiian lomi-lomi (raw salmon), South American ceviche, and pickled anchovies (boquerones en vinagre) and raw sardines in Spain.

Anisakids whose larvae are known to cause human infections include *A. simplex*, *A. physeteris*, *A. pegreffi*, *Pseudoterranova decipiens*, *Contracaecum osculatum*, *Hysterothylacium aduncum*, *Porrocaecum reticulatum*, and *Thynnascaris* spp. Within this group, *A. simplex* and *P. decipiens* are considered the most important human parasites. The term anisakiasis refers to infections with the genus *Anisakis*, anisakidosis refers to infections with members of the family *Anisakidae*, and pseudoterranovosis refers to infections with the genus *Pseudoterranova* (91).

Life Cycle and Morphology

The primary hosts of *Anisakis* are dolphin, porpoise, and whale; those of *Pseudoterranova* are seal, fur seal, walrus, and sea lion. These sea mammals ingest third-stage larvae, which penetrate the gastric mucosa and develop into adult male and female worms. The worms live in clusters, with their anterior ends embedded in the gastric wall. Eggs are then passed out into the sea, where the second-stage larvae are ingested by small marine crustacea (krill) and develop into third-stage larvae. These are then transmitted from krill to fish or from fish to fish, etc., via the normal food chain. The third-stage larvae migrate into the viscera and peritoneal cavity. However, migration into the fish musculature may depend on environmental conditions and/or the species of parasite and fish. They are often transferred from fish to fish along the food chain, and as a result, some fish may amass large numbers of larvae. More than 150 species of fish can serve as intermediate hosts. Herring, salmon, mackerel, cod, and squid tend to transmit *Anisakis* infection, while cod, halibut, flatfish, greenling, and red snapper can transmit *Pseudoterranova*.

Human infection is acquired by the ingestion of raw, pickled, salted, or smoked saltwater fish or squid (Fig. 14.28 and 14.29). The larvae often penetrate into the walls of the digestive tract (frequently the stomach), where they become embedded in eosinophilic granulomas (Fig. 14.30). Occasionally, the throat is involved. These large larvae (third stage) measure 1 to 3 cm long or more by 1 mm wide. Histologic sections are characterized by the large body size, moderately thick cuticle, and large lateral cords that extend into the body cavity.

Clinical Disease

The clinical manifestations of anisakiasis are varied, depending on the site of penetration of the larvae. There may be acute gastric presentation, which is the most commonly recognized clinical syndrome. Untreated gastric disease may produce chronic, ulcer-like symptoms and can be more difficult to diagnose. Intestinal anisakiasis is also seen, sometimes with acute symptoms and sometimes with a mild, chronic presentation; intestinal disease develops within 2 days after infection and occurs most often in the ilial region. Occasionally ectopic disease occurs, where the larvae are found outside of their usual location, usually elsewhere in

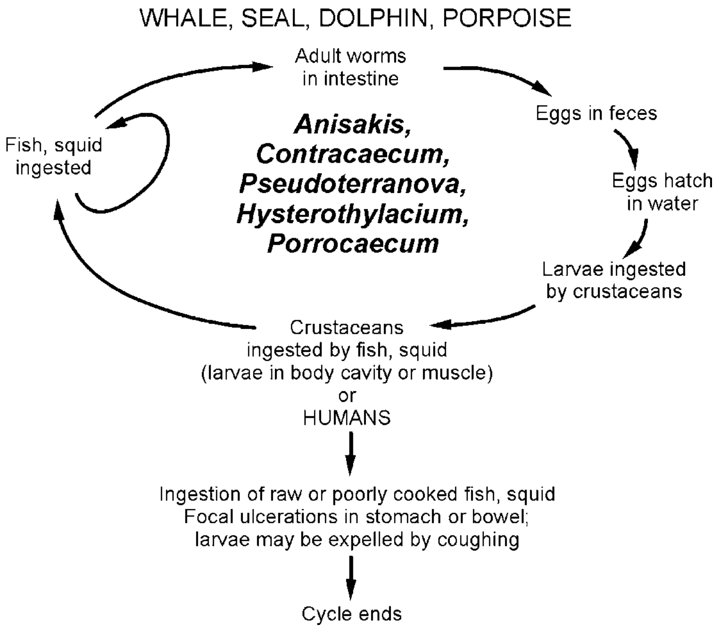
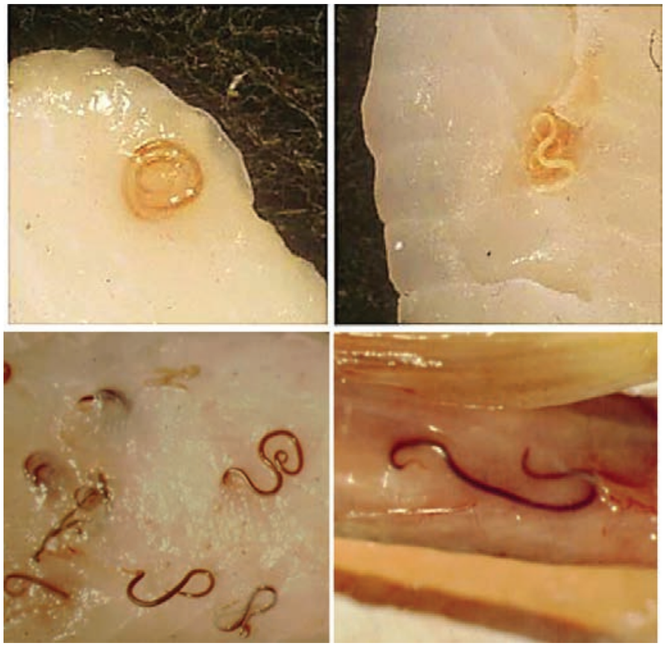

WHALE, SEAL, DOLPHIN, PORPOISE
Adult worms in intestine
Eggs in feces
Eggs hatch in water
Larvae ingested by crustaceans
Crustaceans ingested by fish, squid (larvae in body cavity or muscle) or HUMANS
Ingestion of raw or poorly cooked fish, squid Focal ulcerations in stomach or bowel; larvae may be expelled by coughing
Cycle ends
Fish, squid ingested
Anisakis, Contracaecum, Pseudoterranova, Hysterothylacium, Porrocaecum

Figure 14.28 Life cycle of *Anisakis*, *Contracaecum*, *Pseudoterranova*, *Hysterothylacium*, and *Porrocaecum* spp. (larval nematodes acquired from saltwater fish). doi:10.1128/9781555819002.ch14.f28

Figure 14.29 (Upper left) *Anisakis simplex* in cod; (right) *Pseudoterranova dicipiens* in cod (courtesy of Stig Mellergaard). (Lower left) *Anisakis* in fish flesh; (right) larval nematodes in fish viscera. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of L. A. Jensen.) doi:10.1128/9781555819002.ch14.f29



the gastrointestinal tract. Within North America, one of the most common presentations has been described as the "tingling-throat syndrome" and is often associated with infection by *Pseudoterranova* larvae. In these cases, the patient may even feel the worm in the oropharynx or proximal esophagus. The patient often coughs up the worm, which is then submitted to the laboratory for identification. Larvae in other true ectopic sites are rare, with the abdominal cavity being the most common; mild symptoms are usually the case, and the larvae may be found at surgery for totally unrelated causes. Apparently *P. decipiens* tends to be less invasive than *A. simplex* and is usually expelled by vomiting. *A. simplex* larvae tend to penetrate the gastrointestinal wall, invading the abdominal cavity.

It is well known that *A. simplex* can cause allergic reactions in sensitized patients. At present, a non-seafood diet is recommended for any patients with any kind of *A. simplex* allergy. Symptoms range from urticaria and isolated angioedema to anaphylaxis, and gastrointestinal symptoms can also occur. However, it appears that patients can tolerate the ingestion of seafood when the parasites are dead and noninfective. It has also been suggested that immunologic methods to detect specific antibodies against *Anisakis* should be used routinely before eosinophilic gastroenteritis is diagnosed as the primary disorder. Data indicate that a Th2 mechanism plays an important role in the inflammatory infiltrate produced by the attachment of parasites to the gastrointestinal wall (92).

There may be nausea or vomiting, often within 24 h after ingestion of raw marine fish. Depending on the location of the larvae, infections can mimic gastric or duodenal ulcer, carcinoma, appendicitis, or other conditions requir-

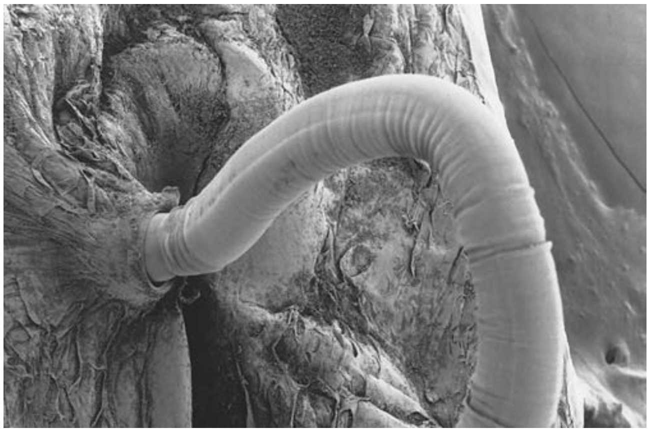


Figure 14.30 Scanning electron micrograph of an *Anisakis simplex* larva penetrating human stomach tissue *in vitro*. Note the tunnel created in the mucosa and the burrow in the submucosa. (Reprinted with permission from Sakanari JA, McKerrow JH, *J Parasitol* **76:**625–630, 1990.)
doi:10.1128/9781555819002.ch14.f30

ing surgery. There is usually a low-grade eosinophilia (10% or less) and a positive result for occult blood in the stool.

There are several reported cases of pulmonary anisakiasis. One of the cases involved a 22-year-old man in Japan, who developed high fever, respiratory distress, and pleural effusion after consumption of raw fish. A parasitic infection was suspected, and various immunoserologic tests were performed. Since extragastrointestinal anisakiasis was strongly suspected, this diagnosis was confirmed by a microplate ELISA and Western blot analysis with a monoclonal antibody (93).

Worms have been recovered or seen after surgery for intestinal obstruction, in eosinophilic granulomas, from a portion of resected small intestine, during gastroscopic examination, in vomitus, and in histologic sections.

Diagnosis

A presumptive diagnosis can be made on the basis of the patient's food history. Definitive identification is based on larval recovery or histologic examination of infected tissue. Although serologic reagents have been developed, they are not commercially available. Molecular biology-based methods may also provide some additional diagnostic tools. A rise in the levels of total and specific IgE in the first month after an allergic reaction, consistent with the patient's history of gastroallergic anisakiasis, can provide valuable information, particularly if the parasite cannot be seen by fiber-optic gastroscopy.

Treatment

There is no recommended therapy other than removal of the larvae, often through surgery (Fig. 14.31). Gastric endoscopy is usually effective in larval location and removal. If the diagnosis is confirmed and there is no ileus, then surgical intervention can be avoided; the larvae will die and become absorbed within several weeks.

Epidemiology and Prevention

Although the distribution of anisakid larvae in infected marine fish is worldwide, within the United States salmon and Pacific rockfish (red snapper) are implicated most often in transmission. Although *Anisakis* normally has a marine life cycle, *A. simplex* and other anisakid parasites have been found in populations of river otter in the Pacific Northwest. This has been linked to the ingestion of shad during their spawning runs and outmigration. Thus, consumption of shad that are infected with anisakid worms may be confirmed as an emerging parasitic disease of veterinary and human medical concern (94).

Raw, pickled, salted, or smoked marine fish should be avoided. All fish intended for raw, partly cooked, or marinated consumption should be blast-frozen to –35°C (–31°F) or below for 15 h or be normally frozen to –20°C (–4°F) or below for 7 days. This disease could be totally prevented by thorough cooking of all marine fish. Also, sushi served at professional sushi bars and restaurants is rarely responsible for infections. Generally in these settings, fish other than salmon, cod, mackerel, herring, whiting, and haddock are used for sushi preparation.

It is also interesting that, even under severe conditions, *P. decipiens* is a well-established parasite of the Antarctic fauna. This cosmopolitan species can complete its life cycle even at subzero temperatures (95).

During the last few years, the finding of allergic hypersensitivity symptoms in anisakiasis has emphasized that this widespread etiologic agent can induce acute symptoms as well as chronic urticaria. Additional reports will certainly confirm the importance of this infection as a potentially growing public health problem (96).

Figure 14.31 *Anisakis*: removal of the worm from the duodenum using biopsy forceps. (Courtesy of Hwang D et al, *Chonnam Med J* **48:**73–75, 2012; open access article http://creativecommons.org/licenses/by-nc/3.0.)
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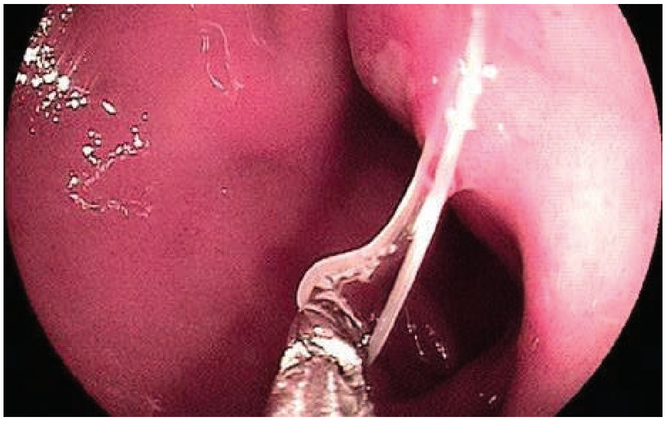


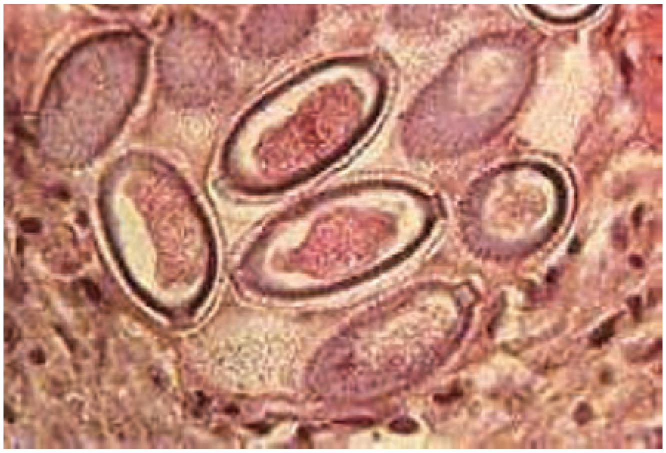


Figure 14.32 *Capillaria hepatica* eggs in liver. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) For information on intestinal capillariasis with *Capillaria philippinensis*, see chapter 13.
doi:10.1128/9781555819002.ch14.f32

Capillaria hepatica

Capillaria hepatica infection is commonly found in rats, other rodents, and other mammals. Human cases have been reported from various parts of the world, including the United States. Infection occurs via accidental ingestion of eggs from the soil. These eggs hatch and are carried via the portal system to the liver, where the larvae mature in approximately 4 weeks and begin to deposit eggs in the liver parenchyma (Fig. 14.32). Symptoms of this infection mimic those of hepatitis, amebic liver abscess, trichinosis, VLM, Loeffler's syndrome, Hodgkin's disease, and histoplasmosis. In the first case reported from Maine, the patient presented with a subacute history of severe abdominal pain, fevers, and weight loss. After open laparotomy for resection of the hepatic mass and treatment with thiabendazole, he recovered; the source of the infection was unknown but was probably accidental ingestion of soil contaminated with mature *Capillaria* eggs (97).

In a true human infection, no eggs are found in the stool. Diagnosis requires histologic examination. Eggs in liver biopsy specimens can be identified on the basis of their characteristic morphology. The recent development of an IFAT may lend itself to testing of human sera for the detection of early *C. hepatica* infection; however, such tests are not available commercially (71).

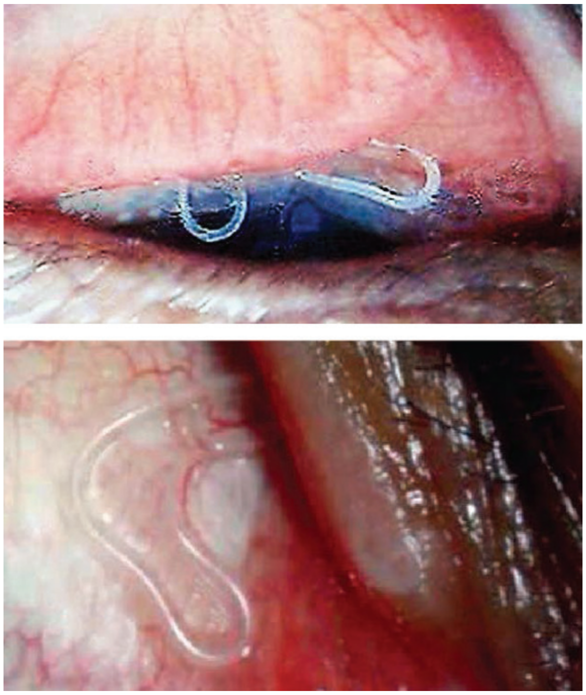
Note In cases of spurious infection, in which infected animal liver has been ingested, *C. hepatica* eggs may be passed in the stool. These eggs measure 51 to 68 μm long by 30 to 35 μm wide and resemble those of *C. philippinensis* (45 by 21 μm), which can be seen in the stool in true human infection (see chapter 13).

Thelazia spp.

Along with *Thelazia californiensis*, which has been reported to infect humans occasionally in the United States, *T. callipaeda* is the only helminth transmitted by secretophagous flies directly into the orbit of humans. Since its first description, this nematode has been known as the "oriental eyeworm" for its distribution in the former Soviet Union and the Asian continent, including China, Korea, Japan, Indonesia, Thailand, Taiwan, and India. *T. californiensis* has been reported from California, and the larvae are transmitted by a fly belonging to the genus *Fannia*.

Human infections may cause mild to severe clinical signs, including lachrymation, epiphora/excess tears, conjunctivitis, keratitis, and/or even corneal ulcers. *Thelazia* spp. have been recovered from the human conjunctiva and may damage the eye tissues. The worms are 1 to 1.5 cm long by 250 to 800 μm wide and are threadlike. They may be found in the conjunctival sac or lachrymal glands or migrating over the cornea (Fig. 14.33). Symptoms are mild and include excessive lacrimation, itching,

Figure 14.33 *Thelazia* spp. The worms are 1 to 1.5 cm long and are threadlike. They may be found in the conjunctival sac or lachrymal glands or migrating over the cornea. (Upper, courtesy of Trang tin điện từ Viện Sõt rét - K Sinh trùng - Côn trùng Quy Nhơn Giãy phép thiẽt lập sõ 53/GP - BC do Bộ văn hóa thông tin cãp ngày 24/4/2005. Lower, courtesy of State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Centre, Sun Yat-sen University, Guangzhou, China.)
doi:10.1128/9781555819002.ch14.f33



or pain (as with a foreign object in the eye). After use of a topical anesthetic, the worms can be safely removed with forceps (27, 35).

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15

Filarial Nematodes

Basic Life Cycle
The Endosymbiont
Human Pathogens
Wuchereria bancrofti
Brugia malayi
Brugia timori
Zoonotic *Brugia* infections (American brugian filariasis)
Tropical pulmonary eosinophilia
Loa loa
Mansonella ozzardi
Mansonella perstans
Mansonella streptocerca
Onchocerca volvulus
Dirofilaria Dirofilaria and *Dirofilaria Nochtiella* spp.

The filarial nematodes are a group of arthropod-borne worms that reside in the subcutaneous tissues, deep connective tissues, lymphatic system, or body cavities of humans. Some adult filarial worms can survive in the human host for many years, causing a number of chronic and debilitating symptoms, including inflammatory reactions. The female worms produce large numbers of larvae called microfilariae, which are highly motile, threadlike prelarvae that in some species maintain the egg membrane as a sheath; these are called sheathed forms, while those that rupture the egg membrane are called unsheathed forms. Once released by the female worm, microfilariae can be detected in the peripheral blood or cutaneous tissues, depending on the species. The microfilariae, which may survive for 1 to 2 years, are not infective for other vertebrate hosts, nor do they undergo any further development in the vertebrate host. The infections are transmitted to humans by the bites of obligate blood-sucking arthropods that become infected through ingesting larvae (microfilariae) contained in a blood meal obtained from a mammalian host (Table 15.1). Each parasite has a complex life cycle, and human infections are not easily established unless there is intense and prolonged exposure to infective larvae. After exposure, it may take years before significant pathologic changes in the human host are evident. The asymptomatic incubation period can be from 6 months to 3 years; therefore, the chances of eliciting a relevant patient history are rare, at best. For this reason, some physicians recommend routine posttravel serologic testing for patients who have been exposed, even years before.

Depending on the species, microfilariae may exhibit periodicity in the circulation. A circadian fluctuation in which the largest number of microfilariae occurs in the blood at night is called nocturnal periodicity; these microfilariae circulate in the peripheral circulation between 9 p.m. and 2 a.m. Some species are nonperiodic or diurnal. In the latter form, the microfilariae circulate at somewhat constant levels during the day and night. Subperiodic or nocturnally subperiodic microfilariae are those that can be detected in the blood throughout the day but are detected at higher levels during the late afternoon or at night. Only a small number of the total microfilarial population is found in the circulating blood, even at peak levels for microfilariae normally found in the blood. When not in the peripheral blood, the microfilariae are found primarily in the capillaries and blood vessels of the lungs. The basis for filarial periodicity is unknown; however, it may be an adaptation to the biting habits of the relevant vector (Table 15.2).

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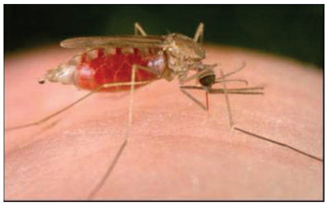
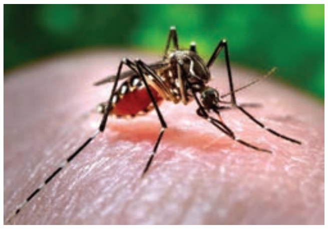
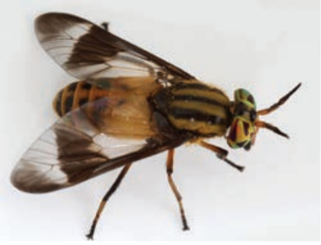
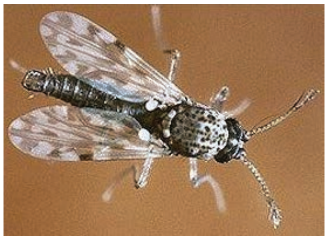
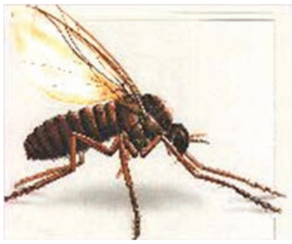
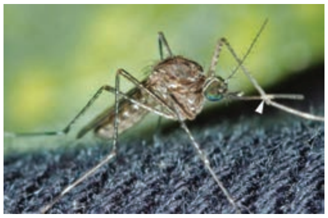
TABLE 15.1 Characteristics of species causing human filariasis

				Features of microfilariae				
Species	**Distribution**	**Vector**	**Adult**	**Location**	**Periodicity**	**Sheath and appearance**	**Length (μm) (range)**	**Tail nuclei**
Wuchereria bancrofti	Tropics and subtropics worldwide; mainly India, China, Indonesia (nocturnal); Eastern Pacific (subperiodic)	Mosquito (*Culex*, *Aedes*, *Anopheles*)	Lymphatic	Blood, hydrocele fluid	Nocturnal, subperiodic	Present; graceful, sweeping curves; blue/pale with Giemsa	260 (244–296)	Do not extend to tip of tail; tail tapers to delicate point; terminal nucleus elongate
Brugia malayi	Southeast Asia, Indonesia, India (nocturnal); Indonesia, Southeast Asia (subperiodic)	Mosquito (*Mansonia*, *Anopheles*, *Coquilletidia*)	Lymphatic	Blood	Nocturnal, subperiodic	Present; stiff, with secondary kinks; pink with Giemsa	220 (177–230)	Subterminal and terminal; tail often constricted between two terminal nuclei; terminal nucleus round
Brugia timori	Islands of Timor and Lesser Sunda in Indonesia	Mosquito (*Anopheles*)	Lymphatic	Blood	Nocturnal	Present; tapering gradually; nearly invisible	310 (290–325)	Subterminal and terminal; no tail constriction between two terminal nuclei; terminal nucleus elongate to oval
Loa loa	Africa	Deerfly (*Chrysops*)	Subcutaneous	Blood	Diurnal	Present	275 (250–300)	Continuous to tip of tail; pointed tail; terminal nucleus elongate
Mansonella perstans	South and Central America, Africa	Biting midge (*Culicoides*)	Body cavities, mesentery, perirenal	Blood	None	Absent	195 (190–200)	Continuous to tip of tail; bluntly rounded tail
Mansonella ozzardi	South and Central America, Caribbean	Biting midge (*Culicoides*), blackfly (*Simulium*)	Subcutaneous, possible body cavities	Blood	None	Absent	200 (173–240)	Do not extend to tip of tail; pointed tail
Mansonella streptocerca	West and Central Africa	Biting midge (*Culicoides*)	Subcutaneous	Skin	None	Absent	210 (180–240)	Single row to tip of tail, tail curved "shepherd's crook"
Onchocerca volvulus	South and Central America, Africa	Blackfly (*Simulium*)	Subcutaneous	Skin	None	Absent	254 (221–287)	Do not extend to tip of tail; finely pointed tail
Dirofilaria immitis	Japan, Australia, United States	Mosquito	Pulmonary nodules (coin lesion); eye	Immature worms have been found in the lungs of humans; thick laminated cuticle, broad lateral ends, large muscle cells; no ridges in the cuticle; eye involvement may be periorbital, subconjunctival, subtenon, or intraocular (pain, redness, blurred vision, swelling of eyelids)				
Other *Dirofilaria* spp.	See comment	Mosquito	Subcutaneous tissues	*D. tenuis* is found in raccoons, *D. repens* is found in dogs and cats; subcutaneous dirofilariasis has been found in the United States, Africa, Asia, Europe, and South America; rare gravid *D. tenuis* worms have been found in humans; thick laminated cuticle, broad lateral ends, large muscle cells, regularly spaced longitudinal ridges and cuticular laminations				

TABLE 15.2 Human filarial parasites and their vectors[a]

Disease	Parasite	Vector	
Bancroftian filariasis	*Wuchereria bancrofti*	Mosquitoes: *Anopheles*, *Aedes*, *Culex*, and *Mansonia* species This is *Anopheles* sp.	
Malayan filariasis	*Brugia malayi*, *B. timori*	Mosquitoes: *Anopheles*, *Aedes*, *Culex*, and *Mansonia* species This is *Aedes* sp.	
Loiasis	*Loa loa*	Red flies: *Chrysops* species	
Mansonelliasis	*Mansonella ozzardi*, *M. perstans*, *M. streptocerca*	Midges: *Culicoides* species	
Onchocerciasis	*Onchocerca volvulus*	Blackflies: *Simulium* species	
Dirofilariasis	*Dirofilaria* species	Mosquitos: *Culex* species	

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[a] Images courtesy of the CDC Public Health Image Library.

Microfilarial periodicity may be altered by reversing the working and sleeping habits of the host. This reversal may take up to a week to complete. For species exhibiting nocturnal periodicity, it has been noted that the insect vector bites primarily at night, whereas in areas of nonperiodic disease, the vector bites mainly during the day.

Basic Life Cycle

All of the human filarial infections have the same basic life cycle, including five larval molts, three in the insect vector and two in the human host. Infection begins with the bite of an infected arthropod vector.

1. Infective L3 larvae are deposited into the skin or blood of the human via the bite of the vector. Larvae pass through the puncture wound and then reach the lymphatic system.
2. A 6- or 12-month period is required for sexual maturity to occur; during this time, the parasite undergoes two molts and the adult worm develops (L5). The adult worms reside in the lymphatics or the lymph nodes.
3. The adult female worm gives birth to L1 larvae called microfilariae. Up to 50,000 microfilariae per day can be produced by the female worm.
4. Microfilariae are ingested by the arthropod vector; two molts occur, and the infective L3 larvae develop and are capable of infecting the human host.

The Endosymbiont

Infection with *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori* causes lymphatic filariasis, while *Onchocerca volvulus* causes "river blindness," which can lead to severe ocular involvement and blindness. These parasites contain an endosymbiotic alphaproteobacterium of the genus *Wolbachia*; these bacteria are *Rickettsia*-like, matrilineally inherited, obligate intracellular bacteria. They are required for larval worm development and adult worm viability and fertility. *Wolbachia* spp. have also been implicated in the pathogenesis of filariasis, including inflammatory responses related to filarial chemotherapy and death of the parasites. These bacteria have also been linked to the onset of lymphedema and blindness (1–3). Female adult worms carry the largest load of bacteria, most of which are concentrated around the reproductive structures.

Filarial and *Wolbachia* antigens activate the release of proinflammatory and chemotactic cytokines, which induce cellular infiltration and amplification of the inflammatory process. Toll-like receptors (TLRs), especially TLR2 and TLR6 (not TLR4 as originally thought), appear to play a role in this process. The increased levels of vascular endothelial growth factors (VEGF) VEGF-C and sVEGF-A seen in lymphedema patients were reduced following doxycycline treatment, which eliminates *Wolbachia*. In patients with hydrocele, doxycycline therapy also reduced the size of the hydrocele with reduced circulating levels of VEGF-A (4). Repeated and extensive exposure to filarial and *Wolbachia* antigens can lead to scarring and fibrosis in lymphatic filariasis and skin thickening and corneal scarring in onchocerciasis.

Like other bacteria in the family *Rickettsiaceae*, *Wolbachia* is sensitive to the tetracyclines (tetracycline and doxycycline) and to azithromycin and rifampin. The use of anti-*Wolbachia* antibiotics is promising as a means to improve the success of other mass treatment programs (2). In one study using doxycycline treatment on the major cause of lymphatic filariasis, *Wuchereria bancrofti*, an 8-week course was found to be a safe and well-tolerated treatment for lymphatic filariasis, with significant activity against adult worms and microfilaremia (5).

Human Pathogens

There are eight filarial species in which the human is the definitive host, six of which are thought to be pathogenic. *W. bancrofti*, *B. malayi*, and *B. timori* all cause lymphatic filariasis. *Loa loa* causes Calabar swellings and allergic manifestations. *Mansonella streptocerca* causes skin disease. *O. volvulus* causes dermatitis and eye lesions. *Mansonella ozzardi* and *Mansonella perstans* are thought to be nonpathogenic.

An estimated 300 million people, primarily in India, Southeast Asia, and sub-Saharan Africa, live in areas where lymphatic filariasis is endemic, and at least 130 million people are probably currently infected. Globally, approximately 2 billion people are at risk for lymphatic filariasis (6). It is estimated that genital disease is present in 25 million infected men and that lymphedema, or elephantiasis, of the leg is present in 15 million people, most of whom are women. Approximately 30 million people are exposed to onchocerciasis, with probably at least 18 million being infected, 270,000 of whom are blind. Conditions associated with human filariasis have a tremendous impact on the physical health, economic well-being, and quality of life of infected persons and the communities in which they live.

It is interesting that in some areas of endemic infection, people do not accept the mosquito theory of transmission but believe in other physical, spiritual, and hereditary causes of lymphatic filariasis. Manifestations are often treated with herbal preparations that are used orally, smeared on the affected parts after scarification, or given as an enema. Although the etiology is thought to be different, the perception of the developmental process of elephantiasis closely parallels medical understanding.

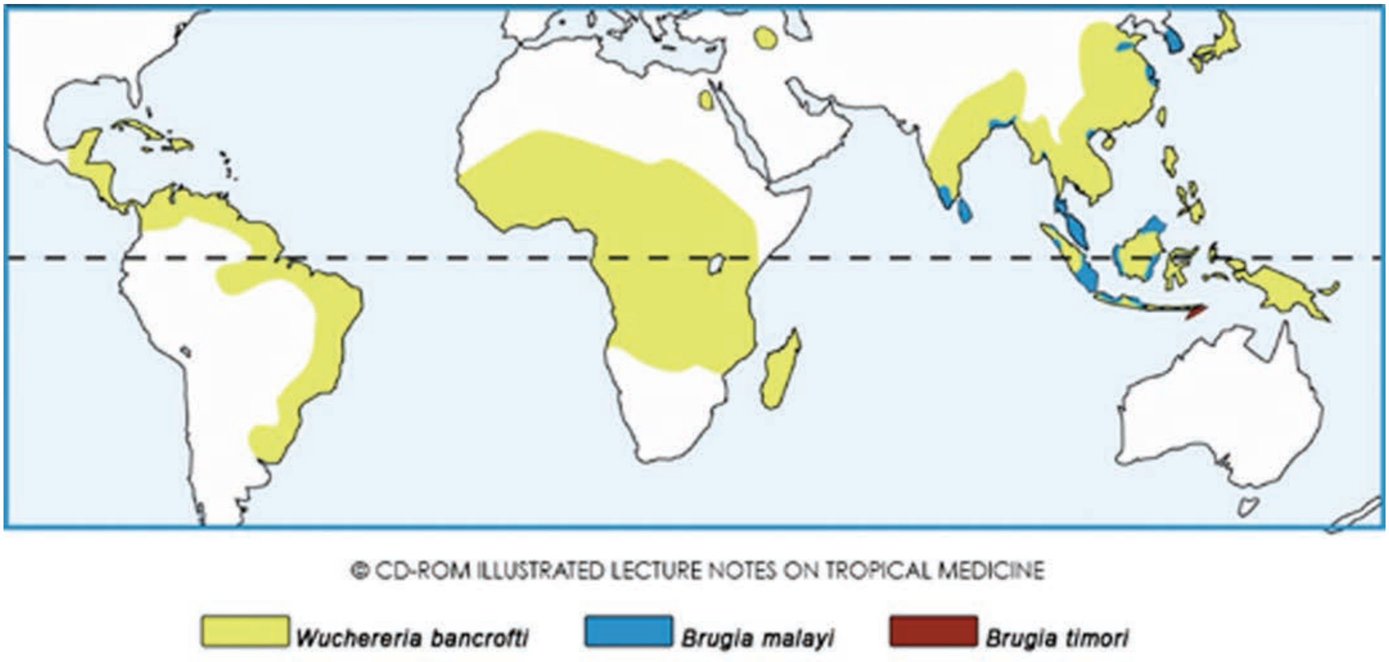
Wuchereria bancrofti

Ancient Egyptian, Hindu, and Persian physicians (600 BCE) were the first to note elephantiasis, which was probably due to *W. bancrofti*. Demarquay discovered microfilariae in hydrocele fluid in 1863, Wucherer found organisms in chylous urine in 1868, Lewis found the microfilariae in blood in 1872, and the adult worm was found in a lymphatic abscess by Bancroft in 1872. By 1900, the entire life cycle of *W. bancrofti* had been elucidated; however, it was Patrick Manson's discoveries that showed for the first time that an arthropod was a vector for a parasitic disease. He had clarified the uptake of microfilariae by *Culex* mosquitoes and their subsequent maturation to infective forms. *W. bancrofti* infections are widely distributed throughout the tropics and subtropics (Fig. 15.1). Humans are the only known reservoir hosts. At one time there was an endemic focus in the region of Charleston, SC, which was probably related to the slave trade.

Life Cycle and Morphology

Humans and mosquitoes are necessary to complete the life cycle of *W. bancrofti* (Fig. 15.2, Table 15.2). The intermediate host, a mosquito, acquires the infection by ingestion of microfilariae in the blood meal. The major vectors in urban and semiurban areas are culicine mosquitoes, while anophelines are involved in the rural areas of Africa and *Aedes* is found in the Pacific Islands. An individual within an area of endemic infection is exposed to approximately 50 to 300 infectious larvae each year. Within hours after their arrival in the mosquito stomach, the microfilariae lose their sheaths. The larvae then penetrate the wall of the gut, migrate to the thoracic muscles, and develop into infective (filariform) larvae over a period of 7 to 21 days. The larvae migrate to the labella (distal end of the proboscis) of the mosquito and enter the skin of the definitive host through the puncture wound when a blood meal is taken. The infective larvae enter the peripheral lymphatic system and migrate to lymph vessels distal to the lymph nodes, where they grow to mature female and male adults and mate. Thousands of developing embryos can be found within the uteri of the female. Microfilariae are released from the gravid female and can be detected in the peripheral circulation 8 to 12 months postinfection; however, filariasis without microfilaremia is not uncommon.

Figure 15.1 Distribution of *Wuchereria bancrofti* and *Brugia malayi* infections in humans. (Armed Forces Institute of Pathology map.) doi:10.1128/9781555819002.ch15.f1



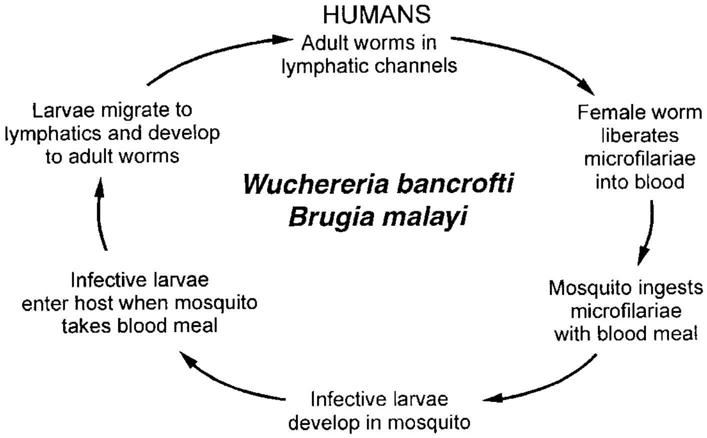

HUMANS
Adult worms in lymphatic channels
Female worm liberates microfilariae into blood
Mosquito ingests microfilariae with blood meal
Infective larvae develop in mosquito
Infective larvae enter host when mosquito takes blood meal
Larvae migrate to lymphatics and develop to adult worms
Wuchereria bancrofti
Brugia malayi

Figure 15.2 Life cycles of *Wuchereria bancrofti* and *Brugia malayi.* doi:10.1128/9781555819002.ch15.f2

Adult worms are minute and threadlike, have a smooth cuticle, and are found in the lymph nodes and lymphatic channels. Adult males are about 40 mm long by 0.1 mm in diameter. Adult females are 80 to 100 mm long by 0.24 to 0.30 mm in diameter. To perpetuate their life cycle, sheathed microfilariae invade the blood; sometimes they can also be found in hydrocele fluid and chylous urine. The microfilariae range from 244 to 296 µm long and actively move about in the lymph or blood. The microfilaria has a sheath, and the body nuclei do not extend to the tip of the tail (Fig. 15.3 to 15.6). Stains such as Giemsa, Wright's, or Delafield's hematoxylin have been used to help differentiate morphological features and thus identify the microfilariae to the species level. Stained microfilariae are about 245 to 300 µm long.

In most regions of the world where filariasis is endemic, *W. bancrofti* microfilariae exhibit periodicity, either nocturnal or subperiodic. In areas of nocturnal periodicity, the microfilariae are in their highest concentrations in the circulating blood at night, generally between 9 to 10 p.m. and 2 to 4 a.m., and are scant or absent during the daylight hours. A subperiodic form of filariasis occurs in the Pacific region, where humans exhibit a microfilaremia all the time but the largest numbers are detected between noon and 8 p.m.

Clinical Disease

Lymphatic Disease. Estimates of 82 to 142 days for the prepatent period have been obtained from experimental human infections, and manifestations of filariasis can range from none to severe, depending on host factors and parasite strains (Algorithm 15.1). Early symptoms of filariasis include high fevers (filarial or elephantoid fever), lymphangitis, and lymphadenitis. Filarial fever usually begins with a high fever and chills that last 1 to 5 days before spontaneously subsiding, and in many cases, patients with filarial fevers do not have a microfilaremia. The lymphangitis extends in a distal direction from the affected nodes where the filarial worms reside (Fig. 15.7). Lymphadenitis and lymphangitis develop in the lower extremities more commonly than in the upper. However, in some areas of endemic infection such as northeastern Brazil, factors other than filarial worms can be the cause of subclinical pathology of the leg lymphatics and are not specific for bancroftian filariasis. In addition to limbs, there can be genital (almost exclusively a feature of *W. bancrofti* infection) and breast involvement. The lymph nodes most often affected are the epitrochlears and femorals. The nodes are firm, discrete, and tender and tend to remain enlarged, while the lymph vessel is indurated and inflamed. The overlying skin is tense, erythematous, and hot, and the surrounding area is edematous. Occasionally, abscesses form at the lymph node or along the lymphatic system and may take 2 to 3 months to heal.

In lymphatic vessels that harbor the worms, an inflammatory reaction includes the infiltration of plasma cells, eosinophils, and macrophages in and around the affected vessel. Repeated inflammatory attacks lead to hyperplasia of the endothelium in addition to cellular infiltration. There is an increase in hydrostatic pressure resulting from the damage to the lymph vessel, which causes an increase in vessel wall permeability. The chronic leakage of fluid containing high levels of protein into the surrounding tissues (lymphedema) results in the hard or brawny edema, with thickening and verrucous changes in the skin, also known as elephantiasis (Fig. 15.8). Histologic examination of the skin shows hyperkeratosis and acanthosis, with scarring and a loss of dermal elasticity. Regional lymph nodes proximal to the worms eventually become fibrotic, and the proximal lymph vessels become stenotic and obstructed, leading to the development of collateral lymph channels. Elephantoid tissue is a mixture of lymph and fat in a matrix of fibrotic tissue. There is the danger of secondary bacterial, particularly streptococcal, or fungal infection in patients with lymph stasis. A specific syndrome, acute dermatolymphangioadenitis, is not caused by filarial worms per se but probably results from secondary bacterial infections.

Obstructed Lymphatics. Obstructed genital lymphatics may lead to hydrocele or scrotal lymphedema. In one area of endemicity, approximately 11% of those at risk developed lymphedema (7). Many of these individuals progressed through sequential phases: uninfected, microfilaremic, and amicrofilaremic to irreversible obstructive lymphatic pathology. Early lymphatic damage was noted in 90% of the men at risk presenting with hydrocele, whereas 90% of the women at risk had no detectable clinical signs of filariasis on physical examination.

Lymph Varices. Lymph varices near the scrotal surface are readily seen and may rupture. Obstruction of the retroperitoneal lymphatics may cause the renal lymphatics to rupture into the urinary tract, leading to chyluria. Chyluria is often prominent in the morning and is intermittent. Almost half of the microfilaremia patients have renal abnormalities characterized by proteinuria and hematuria.

Acute Filarial Lymphangitis. Acute filarial lymphangitis occurs when the adult worms die, leading to severe localized inflammation. This syndrome appears to be uncommon in untreated individuals (8). The dead worms may be absorbed, or they may calcify or produce abscesses. The inflammatory reaction evoked by the filarial infection is a result of host reactions to the products of the developing worm or its death. Microfilariae do not appear to be responsible for the major sequelae of lymphatic filariasis. The immune response to infection varies considerably, with the

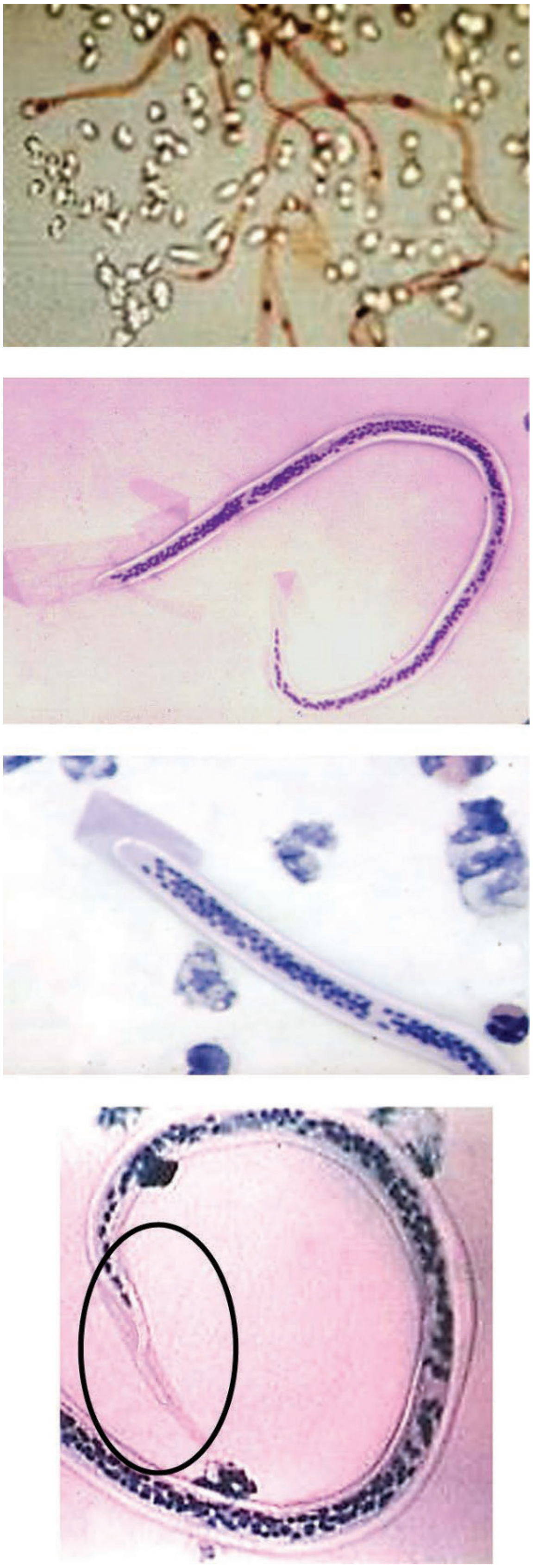


Figure 15.3 *Wuchereria bancrofti*. (Top) Microfilariae on membrane after concentration of venous blood with a Nuclepore filter. (Second) Microfilaria in a peripheral thick blood film. (Third) Head of *W. bancrofti* (note the evidence of a sheath; all images stained using hematoxylin stain). (Bottom) Tail of *W. bancrofti* (note the sheath; also, tail nuclei stop before the end of the tail [oval]). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Bottom three images courtesy of L. Garcia.)
doi:10.1128/9781555819002.ch15.f3

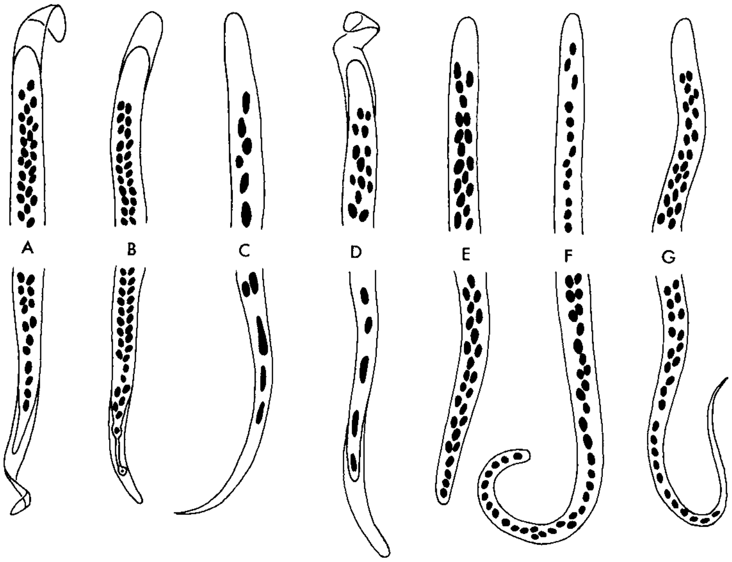


Figure 15.4 Characteristics of human microfilariae. (A) *Wuchereria bancrofti*; (B) *Brugia malayi*; (C) *Onchocerca volvulus*; (D) *Loa loa*; (E) *Mansonella perstans*; (F) *Mansonella streptocerca*; (G) *Mansonella ozzardi*. doi:10.1128/9781555819002.ch15.f4

antibody response being lowest in asymptomatic microfilaremic patients and highest in amicrofilaremic patients. Chronic disease is also strongly associated with acute disease incidence, and microfilaremia has a statistically significant association with acute disease in the leg, arm, and breast but not the scrotum (9).

Amicrofilaremic Patients. Some patients may harbor adult worms without a peripheral microfilaremia, or the microfilaremia may be so low that it cannot be detected by the usual laboratory procedures. Other patients may have a heavy microfilaremia but be clinically asymptomatic. Many individuals who are thought to be microfilaremic but clinically asymptomatic may have symptoms of elephantiasis. Microfilariae are more easily detected in individuals with hydrocele than in individuals with symptoms of elephantiasis. Even though microfilaremia appears to be rare in children younger than 5 years, children born to mothers harboring microfilaria are more likely to be microfilaremic than are children born to afilaremic mothers, suggesting *in utero* acquisition of tolerance.

Figure 15.5 Key to microfilariae commonly found in the blood. doi:10.1128/9781555819002.ch15.f5

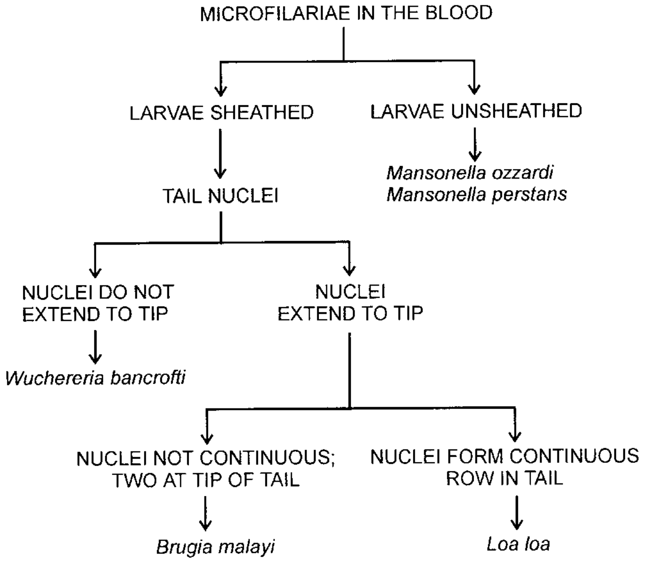
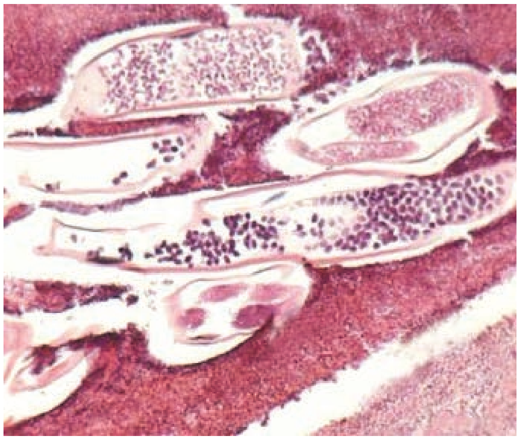
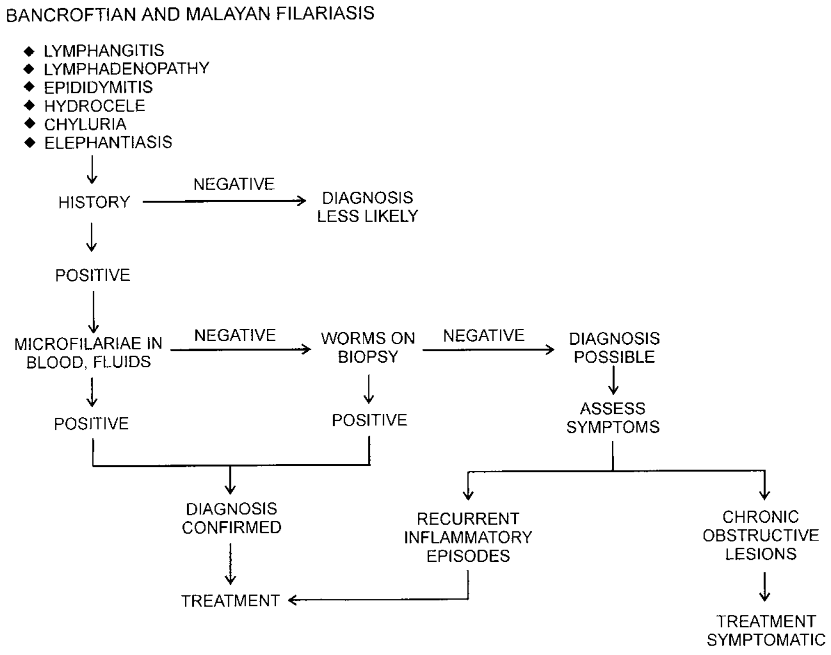

MICROFILARIAE IN THE BLOOD
LARVAE SHEATHED
LARVAE UNSHEATHED
Mansonella ozzardi
Mansonella perstans
TAIL NUCLEI
NUCLEI DO NOT EXTEND TO TIP
NUCLEI EXTEND TO TIP
Wuchereria bancrofti
NUCLEI NOT CONTINUOUS; TWO AT TIP OF TAIL
NUCLEI FORM CONTINUOUS ROW IN TAIL
Brugia malayi
Loa loa

Figure 15.6 *Wuchereria bancrofti* female in lymph node (cross section). (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch15.f6

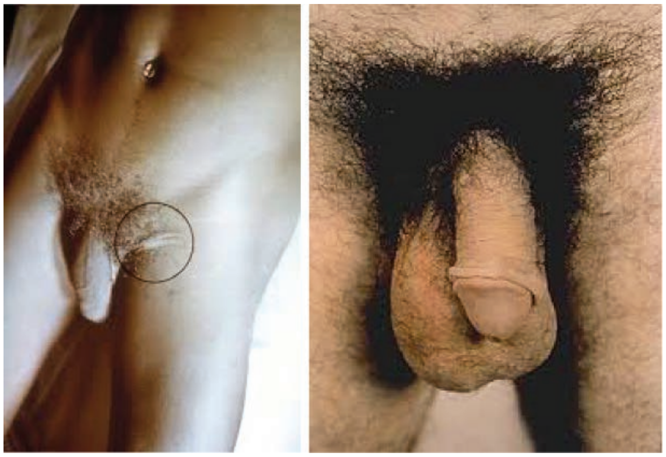



BANCROFTIAN AND MALAYAN FILARIASIS
◆ LYMPHANGITIS
◆ LYMPHADENOPATHY
◆ EPIDIDYMITIS
◆ HYDROCELE
◆ CHYLURIA
◆ ELEPHANTIASIS
HISTORY
NEGATIVE
DIAGNOSIS LESS LIKELY
POSITIVE
MICROFILARIAE IN BLOOD, FLUIDS
NEGATIVE
WORMS ON BIOPSY
NEGATIVE
DIAGNOSIS POSSIBLE
POSITIVE
POSITIVE
ASSESS SYMPTOMS
DIAGNOSIS CONFIRMED
RECURRENT INFLAMMATORY EPISODES
CHRONIC OBSTRUCTIVE LESIONS
TREATMENT
TREATMENT SYMPTOMATIC

Algorithm 15.1 Bancroftian and Malayan filariasis. doi:10.1128/9781555819002.ch15.Alg15.1

Tropical Eosinophilia. Many patients infected with lymphatic filaria do not have microfilaremia. Some may be experiencing tropical eosinophilia (Weingarten's) syndrome. This syndrome is characterized by pulmonary infiltrates, peripheral eosinophilia, cough, asthmatic attacks (especially at night), and a history of prolonged residence in the tropics. These patients have high peripheral eosinophilia counts, high IgE levels, and high antifilarial antibody titers. Microfilariae are not detected in the peripheral blood but may be found in lung biopsy specimens. Chest X rays may be normal but generally show increased bronchovascular changes; diffuse miliary lesions or mottled opacities may be present in the middle and lower lung fields. On treatment with diethylcarbamazine (DEC), there is usually a prompt response and the symptoms disappear.

Figure 15.7 (Left) Inguinal nodes enlarged due to filariasis. (Adapted from the Public Health Image Library, Centers for Disease Control and Prevention.) (Right) Scrotal lymphangitis due to filariasis. (Adapted from the Public Health Image Library, Centers for Disease Control and Prevention.) doi:10.1128/9781555819002.ch15.f7



Infection in Women of Reproductive Age. In a study reviewing pretreatment microfilarial densities, there was a reduction among women of approximately reproductive age; this reduction was statistically significant. However, a comparison of pregnant women and controls showed no evidence that the reduction was specifically related to pregnancy. It has been suggested that the observed reduction in microfilarial intensity may result from hormonal changes associated with female reproduction, possibly in combination with other factors (10).

Immunologic Responses. Asymptomatic microfilaremic individuals generally have a marked Th1 hyporesponsiveness but a strong Th2 response. In contrast, amicrofilaremic individuals with lymphatic pathology have strong Th1 and Th2 responses. There is a decrease in the gamma interferon (IFN-γ) level and a significant rise in interleukin-4 (IL-4) production in infected patients, suggesting a profound form of anergy. In infected individuals, the levels of IgG and IgE are elevated. One of the most consistent find-

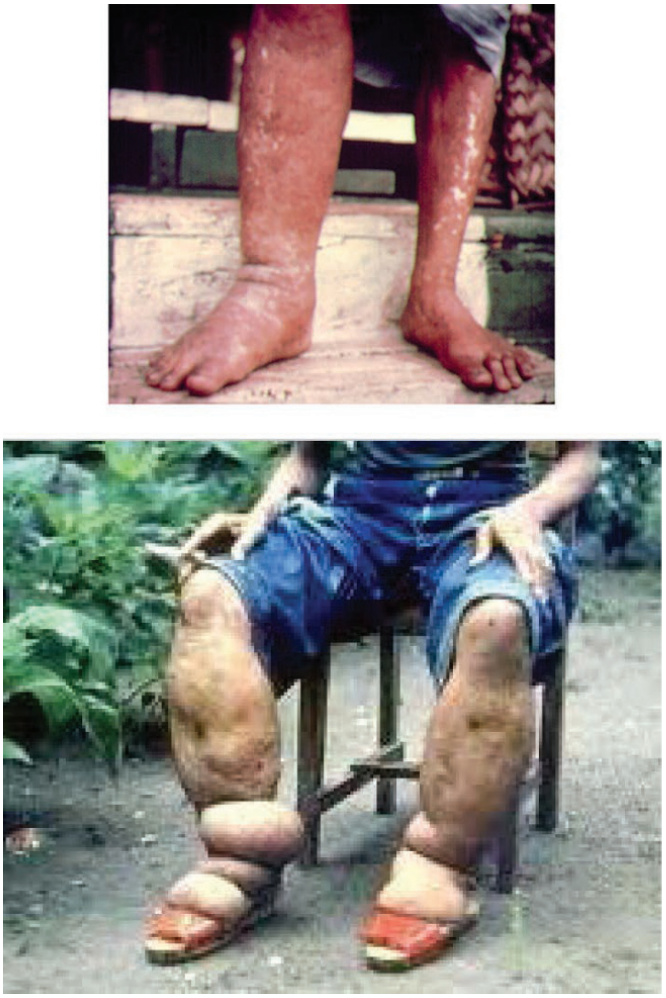


Figure 15.8 *Wuchereria bancrofti* elephantiasis of lower extremities. (Upper, from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Lower, adapted from the Public Health Image Library, Centers for Disease Control and Prevention.)
doi:10.1128/9781555819002.ch15.f8

ings in filarial infections is the elevated level of IgE that is observed following exposure. The highest levels of IgE were found in microfilaremic individuals with acute manifestations of filariasis or hydrocele, while the highest concentrations of parasite-specific IgE were found in amicrofilaremic individuals. This may suggest a protective effect for IgE, because the highest ratios of specific to total IgE are found in patients with tropical pulmonary eosinophilia. The predominant serum antibody response is IgG4. Filarial antigenemia, IgG4 antibody, and household infection are significant risk factors for microfilaremia (11). The ratio of IgG4 to IgE is highest in asymptomatic microfilaremic individuals, whereas chronic disease (elephantiasis) is associated with elevated levels of IgG1 (4). Chronic disease is associated with a specific IgG1 to IgG3 response rather than a Th1 or IgE response. IgG4 levels decline with successful therapy. For signs and symptoms to develop, prolonged exposure to infected mosquitoes is required, and even then the host response is variable.

In French Polynesia, individuals harboring adult worms had a higher filaria-specific IgG4 level but lower IgG3 and IgE levels than did individuals without adult worms, independent of the presence of microfilariae. Low filaria-specific IgG1 and IgG2 levels were associated with the presence of microfilariae but independent of the presence of adult worms. The filarial antibody responses were associated with the parasitological status of individuals but not with clinical symptoms such as hydrocele or limb lymphangitis or elephantiasis. The reduction of filaria-specific immunoglobulin levels was higher after treatment with DEC than with ivermectin, a finding that reflects drug efficacy for the adult worms (12). There appears to be a clear association between IgG2 indirect fluorescent-antibody positivity and a negative microfilarial status and negative specific circulating-antigen status. These findings suggest that specific antisheath antibodies are associated with an immunologic resistance mechanism that is expressed with highest prevalence in young individuals prior to the development of patent microfilaremia.

Children and Adolescents. There is evidence that children acquire infection early in life and that antifilarial antibody responses may peak in early childhood. Apparently, helminth-specific immune responses acquired during gestation persist into childhood, and this prenatal sensitization biases T-cell immunity induced by BCG vaccination away from IFN-γ responses associated with protection against mycobacterial infection.

Often, little attention is paid to the signs and symptoms of bancroftian filariasis in childhood. There appears to be a predominance of lymph node involvement, where the adult worms are located in the vessels of draining lymph nodes, often the inguinal nodes. There are no signs of inflammation in the overlying skin. In the adult population, adult worms are usually found in extranodal lymphatic vessels. Certainly, in areas of endemic infection, filarial infection should be considered in children and adolescents with adenopathy. Ultrasound and other noninvasive methods should be considered; biopsy is probably not necessary (13).

Diagnosis

Routine Blood Tests. Filarial infections should always be considered in the differential diagnosis of individuals who have resided in or migrated from areas where filariasis is endemic and have had a significant insect bite history. In areas of endemic infection around the world, the presumptive diagnosis of filarial infections is frequently based on clinical evidence; however, definitive diagnosis is based on the detection of microfilariae, primarily in the blood. Microfilariae may also be found in hydrocele fluid and urine, particularly in patients who have high microfilaremias or who have been treated recently with DEC.

When a patient is suspected of having filariasis, the clinical history helps determine the most appropriate specimen and collection time. The optimal time for drawing blood to detect nocturnal periodic *W. bancrofti* infections is between 10 p.m. and 4 a.m. Blood used to detect subperiodic *W. bancrofti* may be drawn any time, although peak microfilaremias occur in the late afternoon. Finger prick, earlobe, or venous blood (using EDTA anticoagulant [purple-top tube]) may be taken for direct wet, thin, and thick blood smears. Blood films may be stained with Giemsa or Delafield's hematoxylin stain; however, Giemsa stain does not stain the microfilarial sheath adequately, although hematoxylin stains do so (see chapter 7). Examination of a thin blood film for microfilariae should include low-power review of the entire film, not just the feathered edge. Sheathed microfilariae often lose their sheath when drying on thick films. All thin and thick blood films submitted or prepared for examination for parasites should first be examined using the 10× objective; immediate examination at higher magnifications may miss microfilariae, particularly if in low numbers.

Because of the insensitivity of the wet, thin, and thick blood films, larger blood volumes are recommended for examination to detect low microfilaremias. Also, it may be somewhat impractical to obtain blood from a patient late at night or in the early morning hours. Microfilariae concentrate in the peripheral capillaries; therefore, the numbers of microfilariae in finger prick blood may be similar to those in venous blood. In certain areas, a primary problem with obtaining venous blood is the unwillingness of people to submit to phlebotomy.

Concentration Methods. The Knott technique allows up to 1 ml of blood to be concentrated per 10 ml of 2% formalin so that the sediment can be examined as a wet preparation or stained for microfilariae. Another concentration technique involves the use of polycarbonate filters (Nuclepore) to trap microfilariae on the filter after the red blood cells have been lysed (14) (Fig. 15.3). The 3- to 5-μm-pore-size filters may be examined directly on a microscope slide because the filters are transparent when wet. Infection has been rapidly diagnosed by the detection of microfilaria using a microhematocrit tube coated with acridine orange (QBC method) (see chapter 7). The major disadvantage of the QBC method is the necessity for a fluorescence microscope.

In addition to concentration techniques to detect low levels of microfilariae, a provocative test has been used to induce microfilariae to appear in the blood through treatment with a single dose of DEC. Blood should be drawn within 15 min to 1 h after administration of DEC. A fall rather than a rise in microfilaremia may be detected with the subperiodic strain of *W. bancrofti* after administration of DEC.

Only the microfilariae of *W. bancrofti*, *B. malayi*, and *L. loa* have sheaths; all others are nonsheathed. In addition to the presence or absence of a sheath, the body nuclei should be closely examined for their morphology and location in the tail of the microfilaria. Histopathologic examination of a tissue biopsy specimen may be used for diagnosis to reveal adult worms and microfilariae. Patients who have been treated for lymphatic filariasis should have blood specimens reexamined for microfilariae 2 to 6 weeks posttherapy.

Serologic Methods and Antibody Detection. A wide variety of serologic tests have been used for diagnostic and epidemiologic purposes. Except for patients not native to the area of endemicity, immunodiagnostic tests are of limited value. Current tests lack both sensitivity and specificity, and most people from the region of endemicity have a positive serologic response. This response may be due to exposure to nonhuman filarial antigens from infected mosquitoes; also, filarial antigens may cross-react with antibodies to other parasitic diseases. The immune response of the filaria-specific immunoglobulin IgG4 is considerably increased in active filarial infections. The detection of IgG4 antibodies also reduces the cross-reactivity of nonfilarial antibodies (15). The IgG2 level appears to be increased in patients with elephantiasis. Studies also suggest that specific antisheath antibodies are associated with an immunologic resistance mechanism that in the endemic community is expressed with highest prevalence in young individuals before development of patent microfilaremia (16). Patients with tropical pulmonary eosinophilia have a marked IgE response in addition to elevated eosinophilia.

The recombinant antigen BmR1 has been extensively employed in both enzyme-linked immunosorbent assay and immunochromatographic rapid dipstick (*Brugia* Rapid) formats for the specific and sensitive detection of IgG4 antibodies against the lymphatic filarial parasites *B. malayi* and *B. timori*; however, in sera of individuals infected with *W. bancrofti* the IgG4 reactivity to BmR1 is variable and thus is not recommended for detection (17).

A rapid-format, qualitative flowthrough immunofiltration test has been developed for the identification of total IgG antibodies to recombinant filarial antigen WbSXP-1. This test system employs colloidal gold-protein A reagent as the antibody capture reagent. In a study of 1,230 sera, the sensitivity of the test was 90.8% for patients with proven brugian filariasis (*n* = 70) and 91.4% for patients with proven bancroftian filariasis (*n* = 140). This rapid test is performed with reagents that can be stored at room temperature, is user friendly, and is applicable for field use as an initial screening method. It is also recommended for epidemiologic monitoring of bancroftian and brugian filarial infections (18).

In a new study of 16 antigens, *W. bancrofti* Wb123 was highly immunogenic and specific. Using a rapid luciferase immunoprecipitation system and well-defined sera from normal North Americans and those infected exclusively with intestinal helminths, all of the Wb123-infected individuals (from diverse geographic areas) were detected with 100% sensitivity and 100% specificity (19).

Antigen Detection. Because of the lack of sensitivity of direct-detection methods and problems with obtaining blood specimens within correct time frames, detection of circulating antigens has been used diagnostically. Antigen detection has also been used to monitor therapy. Assays for the detection of circulating antigen of *W. bancrofti* rely on two diagnostic tests, an Og4C3 enzyme-linked immunosorbent assay (TropBio Og4C3 antigen test; TropBio, Townsville, Australia) and an immunochromatographic card test (BinaxNOW Filariasis; Alere, Waltham, MA). The sensitivity of the TropBio test is close to 100%, while the specificity is about 99 to 100%. The ICT has a sensitivity of 96 to 100% and a specificity of 95 to 100% (20, 21). In looking at the possibility of false-positive test results with the BinaxNOW card test, it is clear that the test performs well if the card results are read at the 10-min time limit; false positives may occur if the cards are held and read after 10 min (21). Studies using both of these methods emphasize the benefits of testing to determine the estimation of circulating filarial antigen in a population.

Recently, a new filarial antigen test (the Alere Filariasis Test Strip) was compared with the reference BinaxNOW Filariasis card test that has been used for more than 10 years. Laboratory testing showed that the two tests had similar high rates of sensitivity and specificity and >99% agreement. However, the test strip detected 26.5% more people with filarial antigenemia (124/503 versus 98/503) and demonstrated better test result stability than the card test in a field study conducted in a filariasis-endemic area in Liberia (22).

Molecular Testing. DNA-based techniques have been developed to diagnose and differentiate filarial parasites in humans, animal reservoir hosts, and mosquito vectors. These include DNA hybridization; PCR using specific primers (e.g., SspI repeat, pWb12 repeat, pWb-35 repeat, and LDR repeat for *W. bancrofti* and HhaI repeat, glutathione peroxidase gene, and mitochondrial DNA for *B. malayi*) and universal primers; multiplex-PCR; PCR-restriction fragment length polymorphism (PCR-RFLP); PCR-enzyme linked immunosorbent assay (PCR-ELISA); and quantitative PCR. PCR assays are becoming useful diagnostic tools because they can discriminate between past and present infection and can be used to monitor therapy and to detect and differentiate multiple filarial infections (23). An evaluation of the diagnostic capability of PCR using diurnally collected sputum from patients with bancroftian filariasis has been carried out; the data were excellent, and this approach appears to have great potential (24). A seminested PCR has been used to detect *W. bancrofti* in carriers and in long-term storage blood samples, with no cross-reactivity related to *B. malayi*, *Plasmodium falciparum*, or *Plasmodium vivax* (25). A PCR-RFLP-based method to detect and differentiate a broad range of filarial species in a single PCR has recently been developed. This approach provides differentiation to the species level for *W. bancrofti*, *B. malayi*, *Burgia pahangi*, *Dirofilaria immitis*, and *Dirofilaria repens*. The PCR-RFLP of ITS1 rRNA genes cloned from *W. bancrofti*, *B. malayi*, and *B. pahangi* as universal priming sites will be useful in diagnosing and differentiating filarial parasites in humans, animal reservoir hosts, and mosquito vectors (26, 107).

With the Global Program for the Elimination of Lymphatic Filariasis continuing to pursue disease eradication, many locations where lymphatic filariasis is endemic are seeing a substantial decrease in infection levels and disease transmission rates. However, the need for time-saving and economical molecular assays capable of detecting low levels of parasites is increasing. This is particularly true in areas of endemicity for both *W. bancrofti* and *Brugia* because testing for both causative agents individually results in significant increases in labor and reagent costs. A multiplex, TaqMan-based, real-time PCR assay capable of simultaneously detecting *W. bancrofti* and *B. malayi* DNA extracted from human bloodspots or vector mosquito pools has recently been developed, with significant cost and labor savings (27).

Ultrasonography. Infection with *W. bancrofti* not only affects the structure and function of lymphatic vessels but also is associated with extralymphatic pathology and disease. Since it is now possible to detect living adult worms by ultrasonography, a great deal of emphasis is placed on lymphatic pathology (28). However, the finding of renal damage in asymptomatic microfilaremic carriers has led to increased recognition of the importance of extralymphatic complications in bancroftian filariasis (29, 30). Filarial pathology of the male genitalia is apparently underreported if physical examination alone is used. Compared with ultrasonography, the sensitivity of physical examination in one study was only 44.3% (31). Ultrasonography is also very helpful in assessing lymphatic filariasis in children after treatment and in women (32, 33). In one study, the surprisingly large number of worm nests detectable in microfilaremic women suggests that ultrasonography would be appropriate for diagnosis and posttherapy monitoring of female patients infected with *W. bancrofti* (33). Often the “filaria dance sign” can be seen; this is a characteristic pattern of movement exhibited by adult worms that has been described as rapid and random.

KEY POINTS—LABORATORY DIAGNOSIS

Filariasis

1. A travel and geographic history should be obtained to maximize the best type of specimen and optimal collection time for the filarial infection suspected.
2. In addition to multiple thin and thick blood films, Knott or membrane concentration techniques should be used to detect microfilariae normally found in the peripheral blood.
3. It is important to examine every portion of the thin and thick blood films; microfilariae are often found at the outside edges or in the original drop from which the thin film was “pulled.” All thin or thick blood films should first be examined using the 10× objective (low power). If immediate examination is undertaken at a higher magnification, the microfilariae may be missed, particularly if the parasite numbers are low.
4. Giemsa stain does not stain the *W. bancrofti* sheath as well as a hematoxylin-based stain (Delafield’s hematoxylin).
5. Serologic tests are more meaningful in patients who have not resided in the areas of endemicity for extended periods.
6. Antigen detection tests are commercially available and may be very helpful in the detection of circulating filarial antigens.
7. PCR may prove to be valuable in the diagnosis of lymphatic filariasis; however, these procedures are often limited to research facilities. Some testing is now available commercially.
8. Ultrasonography has proven to be very valuable in assessing lymphatic filariasis in both adults and children; this approach can be much more sensitive than a physical examination alone.

Treatment

It is recognized that some patients with lymphatic filariasis may be asymptomatic; however, if they have microfilariae in the blood, they probably have subclinical disease. Early treatment of these patients is recommended to prevent additional disease manifestations such as lymphatic damage. For patients who appear to have no circulating microfilariae but have a confirmed presence of adult worms, treatment with DEC and albendazole is recommended (34).

Diethylcarbamazine (DEC). The two important drugs in the treatment of filariasis are DEC and ivermectin, which may be used in combination with albendazole (see chapter 36) (32, 35). Treatment differs depending on the objective: interruption of transmission by microfilaria suppression, or treatment of an individual patient. DEC, a piperazine derivative, rapidly kills microfilariae and can kill some but not all adult worms. The effect on adult worms is apparently related to the total dose given, with spaced doses over a period of months to years probably being superior to a more concentrated dose. DEC can be administered orally. Side effects from rapid destruction of microfilariae and possibly adult worms include fever, urticaria, and lymphangitis. These reactions may be controlled by administering antihistamines or by giving low initial doses of DEC and gradually increasing the dose. Nonspecific adverse reactions to DEC treatment include headache, nausea, vomiting, generalized malaise, and vertigo. These symptoms develop within a day or two and may last for several days. A wide variety of treatment regimens have been used to optimize the antifilarial activity of DEC (36, 37). In areas of endemicity, prolonged low-dose administration has successfully reduced transmission and pathologic sequelae. Drug combinations containing DEC are the most effective against microfilarial prevalence and intensity relative to single drugs or other combinations (38).

Ivermectin. Ivermectin (Mectizan; 22,23-dihydroavermectin B_1) is a semisynthetic macrocyclic lactone derivative of avermectin. The drug is taken orally and has been successfully used either alone or in combination with DEC to treat *W. bancrofti* infections (39). The mechanism of action involves chloride ion permeability. Few severe side effects, good microfilaricidal activity, and single-dose oral administration have encouraged its use. In individuals with higher microfilaremia levels, more severe side effects include fever, headache, myalgia, sore throat, and cough. Although there is a rapid and complete clearance of microfilariae in patients treated with one dose of ivermectin in contrast to those treated with DEC, the microfilaria counts begin to rise again after a few months. The rate of increase varies and may be related to differences in the parasite or host responses. At higher therapeutic doses, ivermectin may have cidal activity against the adult worms. It appears that albendazole given in combination with ivermectin as a single dose is more effective in clearing microfilariae than is ivermectin alone and also has the benefit of reducing intestinal helminth infections (40). A significant decrease in the prevalence of *W. bancrofti* infection in mosquitoes has been seen following the addition of albendazole to annual ivermectin-based mass treatments in Nigeria (41).

Tissue Removal and Hydrocele Drainage. Surgical removal of elephantoid tissue has been satisfactory for scrotal elephantiasis but not for effects on the extremities. The use of elastic pressure bandages may help to reduce the induration, but the underlying fibrosis is unaffected. Hydrocele drainage can be repeated or managed surgically; drug treatment should be emphasized for patients with active infection.

Posttherapy Allergic Responses. Microfilaricidal chemotherapy with either DEC or ivermectin induces an acute inflammatory reaction due to an IL-5-dependent increase of eosinophil numbers, eosinophil degranulation, and elevated levels of proinflammatory cytokines such as IL-6, tumor necrosis factor alpha, and IFN-γ. Long-term effects of drug-induced clearance of microfilariae include a reversal of the parasite-specific hyporesponsiveness with restoration of Th1 cell-mediated responses and resistance to reinfection (42). Also, high levels of nitrite and nitrate in serum are released during microfilarial clearance, and sustained elevated levels have been observed 6 months after chemotherapy, suggesting a role of nitric oxide in the elimination of microfilariae. There is also evidence to indicate that although microfilaremic individuals may regain the ability to produce IFN-γ to parasite antigens posttreatment, they subsequently become hyporesponsive to microfilaria-containing antigens; this appears to be independent of the recurrence of microfilaremia and the response to nonparasite antigens (43).

Epidemiology and Prevention

W. bancrofti infections are widely distributed throughout the tropical and subtropical regions of Africa, Asia, Central and South America, the Caribbean islands, and the Pacific Islands. This species infects an estimated 115 million individuals; however, based on diagnostic test limitations, the actual number may be twice as great. *Anopheles* and *Culex* mosquitoes are night-biting vectors for the nocturnally periodic *W. bancrofti*, while the subperiodic strain is transmitted by day-biting *Aedes* mosquitoes. In areas of endemicity, exposure begins early in childhood, with microfilaria rates increasing with age, although the infection may not be clinically apparent.

Research on lymphatic filariasis has mainly focused on rural environments, where it also has its major impact. However, there is also a potential for urban transmission, which has been identified as one of the major future challenges (44). *W. bancrofti* has a significant potential for urban transmission; one of its vectors, *Culex quinquefasciatus*, thrives and reproduces in crowded city areas with poor sanitary, sewerage, and drainage facilities. There do not seem to be any major differences in the pattern of infection and disease prevalence between the surveyed urban sites and that seen in rural areas.

Since these helminths do not multiply within the human host, infection levels are related directly to the number of infective larvae to which humans are exposed. Thus, prevalence and infection levels in an area of endemicity depend on the intensity of transmission. Factors that influence transmission include vector breeding sites, parasite longevity, levels of microfilariae in the blood when the vector bites, microfilarial load in the population, prevalence of lymphatic pathology, and immunologic tolerance. Transmission is quite inefficient, and nonresidents who move into areas of endemicity rarely develop patent infections; however, they may develop signs of acute disease (45).

Individual protection involves the use of insect repellents and bed netting treated with long-lasting insecticide; however, long-term protection has been sought through vector control and the use of chemotherapy. To carry out vector control properly, one must identify the species responsible for transmission, including its feeding, flight, and breeding habits. Because identification of filarial species in infected vectors by microscopy is difficult, DNA hybridization has been used to help identify mosquito populations involved in transmission. Wet pit latrines are favorite breeding sites for mosquitoes, and sanitation improvement to reduce breeding places in urbanized communities where the infections are endemic is a very effective means of control. Although vector control and mass treatment have been successful in small isolated areas, these approaches have been difficult in large areas.

In areas of endemic infection, DEC has been given to the inhabitants at weekly and monthly intervals over long periods to reduce transmission. The drug has even been incorporated into table salt for continuous low-dose administration to the population. Unfortunately, substantial increases in microfilaremia in humans and higher infection rates in mosquitoes in these areas have been noted when mass DEC chemoprophylaxis has been stopped.

Ivermectin inhibits the exsheathment of microfilariae in mosquitoes. This action prevents biological development within the mosquito, thereby disrupting the chain of transmission. Vaccine development strategies have focused on unique filarial proteins that are critical for biological functions; however, there are no vaccines in general use today (46).

Brugia malayi

Microfilariae of *B. malayi* were first observed by Lichtenstein in blood films from natives in Indonesia, and Brug described the microfilariae as a new species in 1927. Rao and Maplestone recovered adult worms from the forearm of a patient in India in 1940, giving the first descriptions of *B. malayi* adults. The geographic distribution of *B. malayi* overlaps that of *W. bancrofti* in certain areas (Fig. 15.1). Both species are considered to be lymphatic filariae; however, *Brugia* spp. are much more widely adapted to animal hosts other than humans.

Life Cycle and Morphology

Although the two species can be differentiated morphologically, the life cycle of *B. malayi* is similar to that of *W. bancrofti* (Fig. 15.2). However, *Brugia* has a shorter development time in the mosquito vector and the time from infection to appearance of microfilariae may be as short as 3 to 4 months (Table 15.2). The adult worms inhabit the lymphatics, and the females give birth to sheathed microfilariae. The microfilariae differ from those of *W. bancrofti* by having two terminal nuclei that are distinctly separated from the other nuclei in the tail (Fig. 15.4 and 15.5). The last terminal nucleus is quite small and is found at the tip of the tail (Fig. 15.9). The microfilariae range from 177 to 230 μm in length. The intermediate host is a mosquito that may be infected with a periodic or subperiodic strain, depending on the geographic area.

Clinical Disease

The clinical pathology of *B. malayi* infections in humans is similar to that of *W. bancrofti.* Clinical manifestations usually develop months or years after infection, and many of the patients are asymptomatic even when they have microfilaremia. Lymphangitis and filarial abscesses occur with a greater degree of frequency than in *W. bancrofti* infections. If elephantiasis occurs, the swelling is normally restricted to the lower extremities below the knee. Sclerotic cordlike lymphatics and enlarged nodes in the arms and legs are common; urogenital involvement with chyluria does not occur. In disease caused by *B. malayi*, episodes of prolonged fever, adenolymphangitis, abscesses of affected lymph nodes, and local residual scarring occur quite frequently. Chronic lymphedema or elephantiasis, as seen in bancroftian filariasis, does not occur frequently.

Cytokine analysis revealed that in an area where brugian filariasis is endemic, IFN-γ was most affected by the shifts in microfilarial densities; proliferative responses remained low throughout the study period in microfilare-

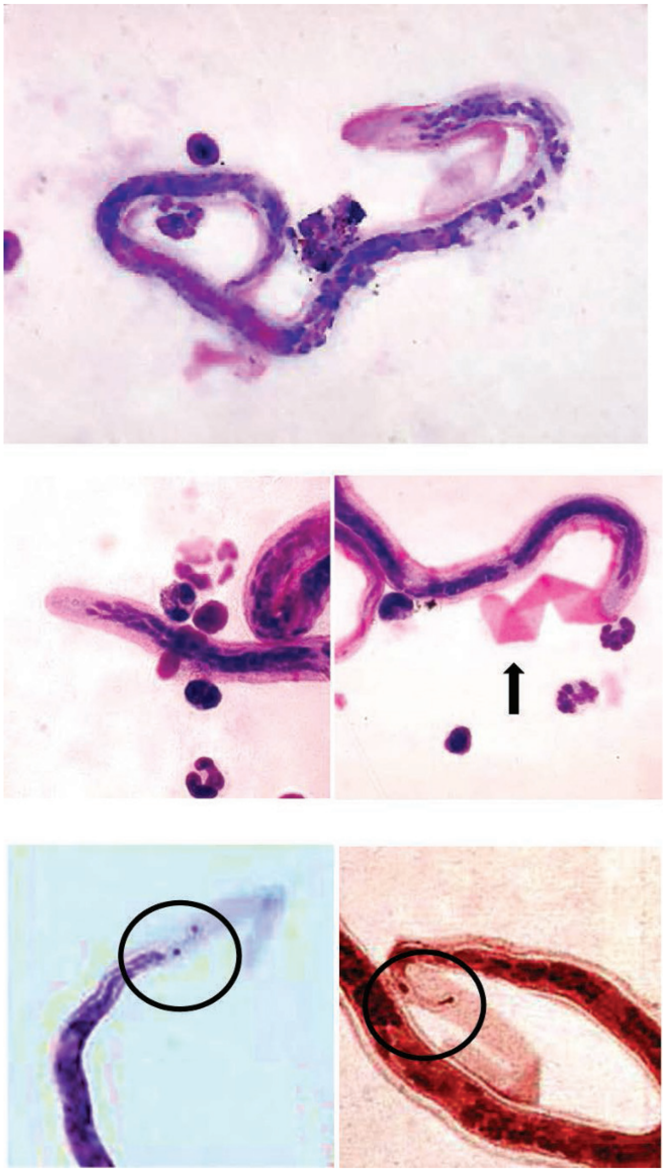


Figure 15.9 *Brugia malayi*. (Top) Microfilaria demonstrating sheath (pink using Giemsa stain). (Middle, left) Head of microfilaria: note that the sheath is not that clearly visible; (right) head showing sheath stained with Giemsa stain (arrow). (Bottom, left) Tail of microfilaria: note the two terminal nuclei separated from the other nuclei (circle); the bulge around the first nucleus is not visible as in the next image; (right) tail of microfilaria: note the sheath and the two terminal tail nuclei, also note the slight bulge around the first tail nucleus (circle). Note the body nuclei are not as precise and separate as they are in *W. bancrofti*. (Courtesy of the CDC Public Health Image Library).
doi:10.1128/9781555819002.ch15.f9

mic individuals; however, in those with no demonstrable microfilariae, the responses were inversely related to the microfilarial densities (47). In contrast, IL-4 responses showed little correlation with changes in parasite densities.

Diagnosis

The diagnostic methods are similar to those for *W. bancrofti* and include thin and thick blood films, wet preparations and concentrations, PCR, ultrasonography, and antigen and antibody detection. There are nocturnally periodic and nocturnally subperiodic strains, so travel history can be helpful in determining optimal specimen collection times. Giemsa stains the sheath of *B. malayi* (will stain pink) but does not stain the sheath of *B. timori*, a species found in the islands near Indonesia. Apparently, the sheath is absent in 50% of periodic microfilariae but in only 5% of subperiodic microfilariae (Fig. 15.4 and 15.5; Table 15.1; Algorithm 15.1). As mentioned under *W. bancrofti* above, a multiplex, TaqMan-based, real-time PCR assay capable of simultaneously detecting *W. bancrofti* and *B. malayi* DNA extracted from human bloodspots or vector mosquito pools has recently been developed, with significant cost and labor savings (27).

Treatment

The frequency of severe reactions is greater in *B. malayi* infections than in *W. bancrofti* infections. Initiation of treatment with lower doses of DEC and use of anti-inflammatory drugs reduces the adverse reactions. Ivermectin has also been used to treat *B. malayi* infections. Side effects are attributed to host responses to the death of microfilariae rather than directly to the drug. Single doses are effective in clearing or reducing the microfilaremic burden for up to 6 months (48). However, a gradual decrease in microfilaria occurs over several weeks to 15 to 20% of pretreatment levels; this response is much slower than that seen with *W. bancrofti* and microfilaria clearance. The combination of DEC and albendazole, both well-tested drugs, offers a new option for countries such as India, where there is no onchocerciasis or loiasis and where ivermectin may not be readily available; the efficacy of albendazole against intestinal helminths would provide additional benefits (49). Since acute attacks of adenolymphangitis (ADL) contribute to the overall morbidity with brugian filariasis, studies now indicate that foot care seems to play the most important role in the prevention of ADL attacks. Benefits may accrue from local or systemic antibiotic use in patients with high grades of edema, whereas antifilarials are ineffective in the prevention of ADL attacks.

Epidemiology and Prevention

There are two types of *B. malayi*, one nocturnally periodic and the other nocturnally subperiodic. The periodic strains, which have no animal reservoirs, are widely distributed in Asia, whereas the subperiodic form has been noted in Malaysia, Indonesia, and the Philippines. The principal mosquito vectors are *Mansonia* spp.; however, in certain areas *Anopheles* and *Aedes* spp. may be important. There are a number of reservoir hosts for *B. malayi* infections, including humans, dogs, cats, and monkeys.

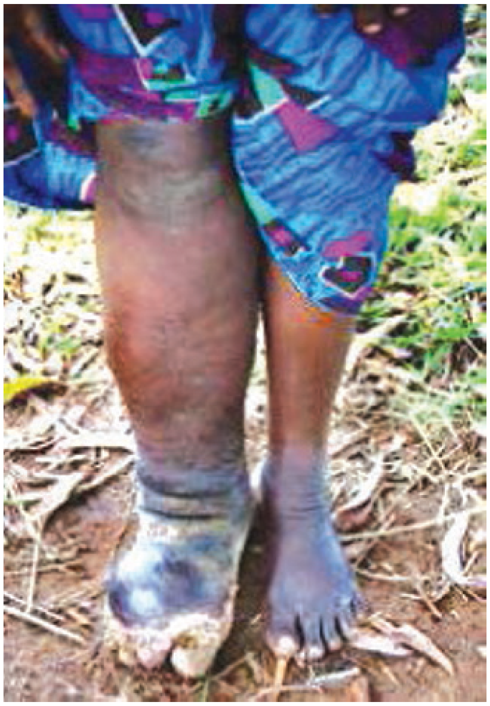
Any prevention program must consider reservoir hosts other than humans in the control of *B. malayi*. Measures used for control and prevention have been similar to those used for *W. bancrofti*.

Brugia timori

Human infections in the Indonesian island chain (Timor) were first reported in 1964, and the filarial worms were recognized in 1965 by David and Edeson as being distinctly different from *B. malayi* (50). *B. timori* infections produce symptoms and pathology similar to those found in *W. bancrofti* and *B. malayi* infections, and the life cycle is similar. However, there appear to be more abscesses related to *B. timori* infections. As with *B. malayi* infections, if elephantiasis occurs, the swelling is normally restricted to the lower extremities below the knee (Fig. 15.10).

The microfilariae have nocturnal periodicity and are transmitted by *Anopheles* mosquitoes. *B. timori* microfila-

Figure 15.10 *Brugia timori* elephantiasis of the lower extremity. (Courtesy of USAID's NTD Program, source Andrea Peterson.) doi:10.1128/9781555819002.ch15.f10



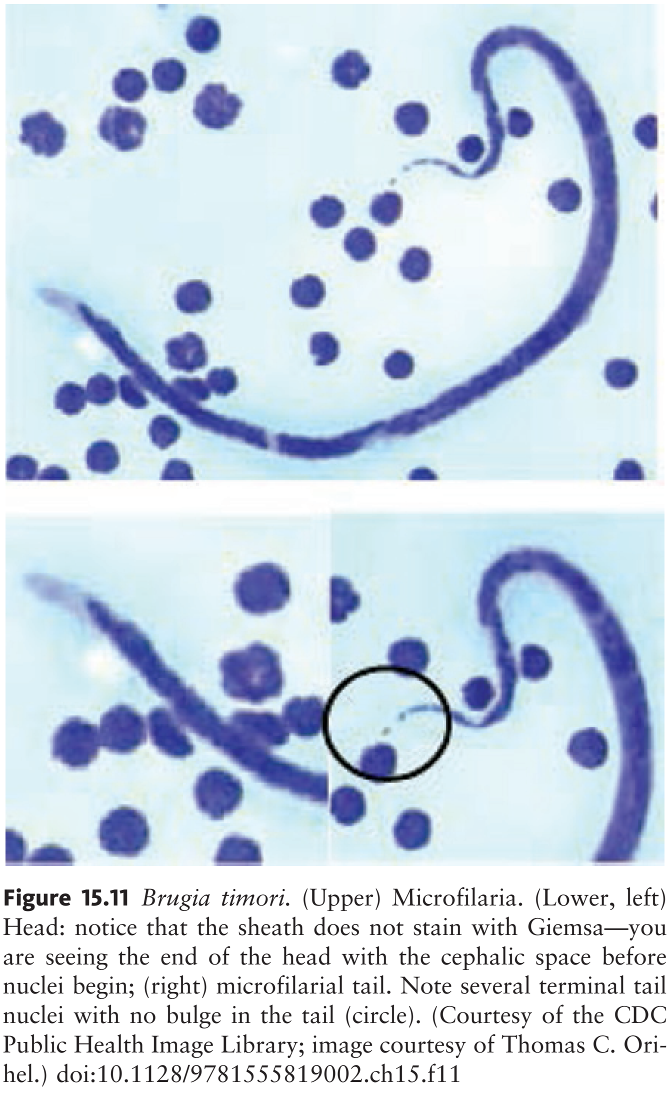


Figure 15.11 *Brugia timori.* (Upper) Microfilaria. (Lower, left) Head: notice that the sheath does not stain with Giemsa—you are seeing the end of the head with the cephalic space before nuclei begin; (right) microfilarial tail. Note several terminal tail nuclei with no bulge in the tail (circle). (Courtesy of the CDC Public Health Image Library; image courtesy of Thomas C. Orihel.) doi:10.1128/9781555819002.ch15.f11

riae are morphologically similar to *B. malayi* microfilariae, except that on average they are longer (310 versus 220 µm), the cephalic space (length from the tip of the microfilaria head to the first body nucleus) has a length/width ratio of 31 versus 21, and the sheath does not stain with Giemsa stain as well as the *B. malayi* sheath does (Table 15.1). Also, there tend to be five to eight, rather than four or five, contiguous nuclei in the tail, and little to no bulge is seen in the cuticle (Fig. 15.11).

The *Brugia* rapid test is an immunochromatographic dipstick test used to detect IgG4 antibodies that are reactive with a recombinant *B. malayi* antigen. This approach has been used successfully for the detection of *B. timori* antibodies and has been recommended as a monitoring tool for *B. timori* in the framework of the Global Program to Eliminate Lymphatic Filariasis (51, 52). Treatment is similar to that for *B. malayi* infections (51).

Zoonotic *Brugia* Infections (American Brugian Filariasis)

Many kinds of filariae have been isolated from humans, including species of *Dirofilaria*, *Brugia*, *Onchocerca*, *Dipetalonema*, *Loaina*, and *Meningonema*. Under proper circumstances, it is probable that almost any filaria parasitizing animals can infect humans and undergo some degree of development. Worms have been found in subcutaneous tissues, the heart and lungs, lymphatics, the eye, and the central nervous system. A number of species of *Brugia* have been implicated in zoonotic transmission from animals to humans worldwide, including the United States. Some species normally infect dogs, cats, rabbits, and raccoons. Human infection with *Brugia* spp. is also referred to as brugian filariasis, zoonotic brugian filariasis, or brugian zoonosis. Mosquitoes may accidentally transmit the infective stages by feeding on an infected animal and then on a human approximately 2 weeks later (53–55). Fewer than 50 cases have been reported, most of which have been documented since 1977. Most of the cases in the United States have been seen in the northeastern part of the country and have been due to *B. beaveri* from the raccoon (*Procyon lotor*) and possibly bobcat and mink, and *B. leporis* from swamp rabbits (*Sylvilagus aquaticus*) and eastern cottontails (*Silvilagus floridanus alacer*) (56). Cases have been reported from Malaysia, where differentiation of this infection from other endemic infections is difficult; reports of infections, most of which are probably due to *B. guyanensis* infecting the raccoon-like coatimundi (*Nasua nasua*) and the weasel-like grison (*Grison vittatus*), have also come from South America, specifically Colombia, Brazil, Ecuador, and Peru.

Patients with American brugian filariasis rarely exhibit any systemic symptoms. Usually patients present with a tender mass in the cervical, axillary, or inguinal region; the mass may have been present for a few weeks before the patient sought medical help. A number of different conditions may be suspected, including an enlarged lymph node, lymphoma, lipoma, and papilloma. Specific areas of the body that have been involved include the pulmonary artery, the groin, the area behind the ear, the chest wall, and the neck. Once the mass is removed and examined histologically, a nematode worm is often found lodged in a dilated lymphatic vessel in the capsule, cortex, or interior of the node. The worm is often living and the structure is well preserved; however, if the worm is dead, it is probably surrounded by a granulomatous reaction. Once the mass is removed, no further treatment is required. *B. beaveri* and *B. leporis* are the only described species known to occur in the United States; they are commonly found in the raccoon in Louisiana, and microfilariae have been recovered from bobcats in Louisiana and Florida.

In Malaysia, *B. pahangi* is a common parasite of dogs and cats and has been implicated in cases of lymphangitis and lymphadenitis. It may be very difficult to differentiate infections with *B. pahangi* from endemic infections with *B. malayi*, particularly since they have the same mosquito vectors and the microfilariae are very similar to those of *B. malayi*.

Tropical Pulmonary Eosinophilia

Tropical pulmonary eosinophilia (TPE) is associated with *W. bancrofti* or *B. malayi* infections causing diffuse pulmonary infiltrates with significant local and systemic eosinophilia and high levels of polyclonal and parasite-specific IgE (45). Migration of microfilariae through the pulmonary blood vessels with subsequent allergic hypersensitivity responses is thought to be the cause of this syndrome. The epidemiology of this condition is not well understood, particularly since there can be other causes. It seems to occur in long-term residents but not in travelers or residents who have been in the area of endemicity for a short time or only a few years. TPE can also occur many years after susceptible individuals have left the area. The majority of cases have been reported in Brazil, Guyana, India, Pakistan, Sri Lanka, and Southeast Asia.

Studies have shown numerous pulmonary granulomas, possibly indicating trapping of the parasites within the lungs. Because no blood-borne microfilariae are present in these patients, the syndrome may be due to an intense inflammatory response to the migration of microfilariae through the pulmonary blood vessels. No apparent reason for this outcome in a small number of patients has yet been confirmed.

TPE usually occurs in young men, and the onset is gradual, progressing over a period of months. Patients present with a nonproductive cough, wheezing, fever, generalized malaise, fatigue, and weight loss. Symptoms tend to occur at night and may be associated with the release of microfilariae that have become trapped in the lungs. The chest X ray usually shows diffuse interstitial or reticulonodular infiltrates 1 to 3 mm in diameter; increased bronchovascular markings are also seen.

Diagnosis is based on clinical, epidemiologic, and laboratory findings. Successful resolution of symptoms after treatment with DEC generally confirms the diagnosis; the same dosage schedule as for bancroftian filariasis is used. Failure to respond to DEC strongly suggests another diagnosis, including allergic granulomatosis with angitis, Wegener's granulomatosis, periarteritis nodosa, allergic bronchopulmonary aspergillosis, idiopathic hypereosinophilic syndrome, and severe asthma. TPE is an important diagnostic consideration in patients with eosinophilia, respiratory symptoms, and a history of exposure to this disease. Without therapy, TPE can lead to chronic and progressive respiratory disease and then to death. Prompt recognition and treatment with DEC are critical to patient outcome (58).

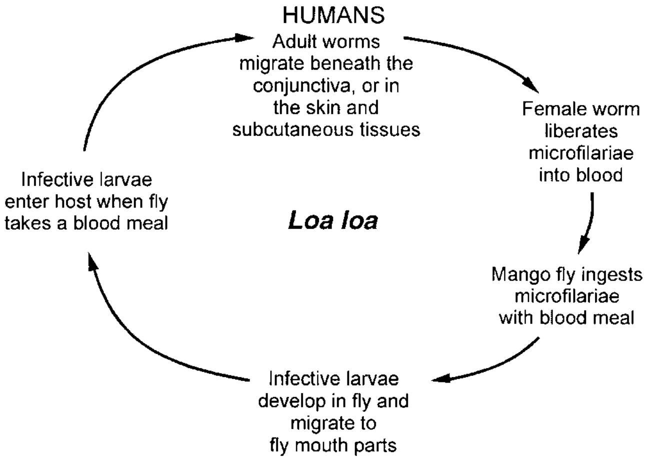
Loa loa

Loa loa, the African eye worm, was first noted in the eye of a native girl in the West Indies in 1770. In 1895, Argyll-Robertson described the adult worms that he extracted from the eye of a woman who had resided at Old Calabar in West Africa. The adult worms migrate through the subcutaneous tissues, producing intermittent "Calabar swellings," in addition to migrating beneath the conjunctiva. Approximately 13 million people are infected with *L. loa* in Central and West Africa. The vectors are biting flies (*Chrysops*) and are known as red flies in Africa.

Life Cycle and Morphology

The adult male and female worms live and migrate in the subcutaneous and deep connective tissues, and the microfilariae are found in the blood, where they can be ingested by mango flies or deerflies (*Chrysops* spp.) (Fig. 15.12, Table 15.2). Once ingested by the fly, the microfilariae of *L. loa* lose their sheaths, penetrate the gut wall, migrate to the fat body, undergo two molts, and become infective in approximately 10 to 12 days. Humans are infected when bitten by infected flies, and the infective larvae enter the skin through the bite wound. Development into adult worms takes about 6 to 12 months, and they can survive up to 17 years. The females measure 50 to 70 by 0.5 mm, while the males measure 30 to 35 by 0.3 to 0.4 mm. The microfilariae frequently are not detected in the blood until years after the adult worms are noted. The microfilariae

Figure 15.12 Life cycle of *Loa loa*. doi:10.1128/9781555819002.ch15.f12


HUMANS
Adult worms migrate beneath the conjunctiva, or in the skin and subcutaneous tissues
Female worm liberates microfilariae into blood
Mango fly ingests microfilariae with blood meal
Infective larvae develop in fly and migrate to fly mouth parts
Infective larvae enter host when fly takes a blood meal
Loa loa

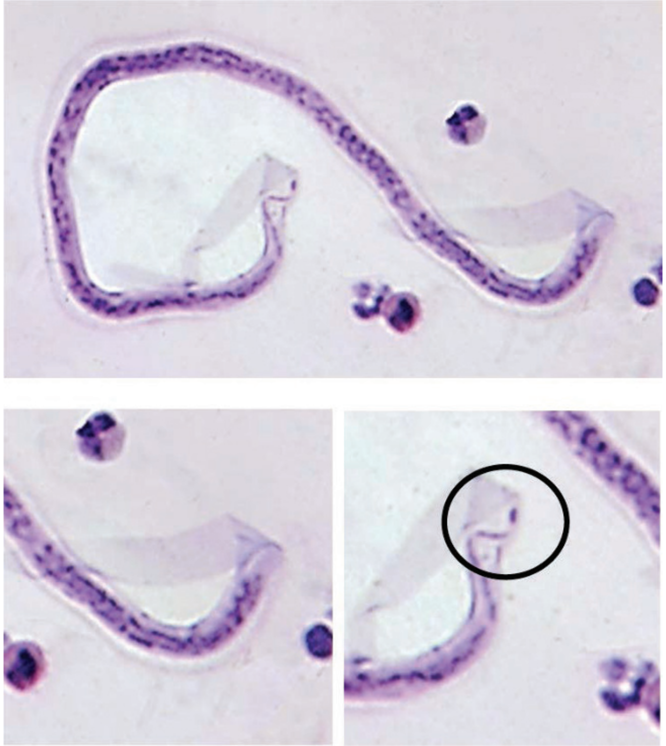


Figure 15.13 (Upper) *Loa loa* microfilariae in blood. Note the presence of the sheath and the tail nuclei positioned to the end of the tail. (Lower, left) Head of *Loa loa*: note the sheath stained with Giemsa stain; (right) tail of *Loa loa*: note the sheath and the terminal nucleus at the tip of the tail (circle).
doi:10.1128/9781555819002.ch15.f13

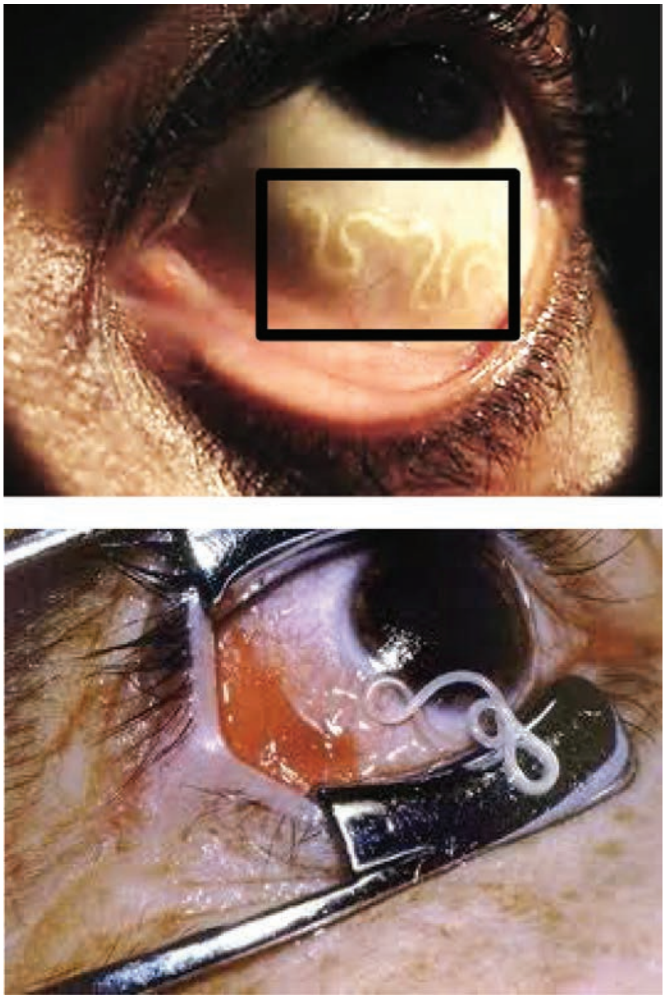


Figure 15.14 (Upper) *Loa loa*. Subconjunctival nematode (box). (Lower) *Loa loa* being removed from the eye. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of D. Gendelman.)
doi:10.1128/9781555819002.ch15.f14

have a diurnal periodicity whose peak occurs about midday; the remainder of the time they can be found in pulmonary capillaries. The microfilariae are sheathed and are 250 to 300 µm long. When stained, the body nuclei are continuous to the tip of the tail (Fig. 15.4, 15.5, 15.13, and 15.14). The developmental cycle in the mango fly or deerfly is similar to the development of *W. bancrofti* in the mosquito.

Clinical Disease

The *Chrysops* bite results in erythema, swelling, and itching, symptoms which can worsen with the presence of infective larvae (Table 15.2). The adult worms normally migrate through the subcutaneous tissue at a rate of about 1 cm/min. This migration is not painful and is seldom noticed unless the worm is passing over the bridge of the nose or through the conjunctiva of the eye (Fig. 15.14). Many patients with active *L. loa* infections do not have a microfilaremia. The most common pathologic sequelae associated with *L. loa* infections are Calabar or fugitive swellings (angioedema). Calabar swellings are localized subcutaneous edemas, a type of inflammatory reaction brought about by a host response to the worm or its metabolic products. Calabar swellings may be found anywhere on the body but predominate on the extremities. The swellings develop rapidly over a few hours and may be preceded by localized pain, pruritus, and urticaria; the swellings usually last 1 to 3 days. Serious complications due to loiasis have included cardiomyopathy, encephalopathy, nephropathy, and pleural effusions. Even with low doses of DEC to initiate treatment of patients from the area of endemicity, followed by gradually increasing doses combined with corticosteroids, microfilaremia encephalitis is frequently fatal (59). Host genetic factors appear to be involved in acquiring loiasis in areas of endemic infection, because individuals who are not microfilaremic by the age of 30 have a low probability of becoming positive later in life (60). A Th2-type immune response has been found in microfilaremic *L. loa*-infected patients (61), and IgE antibodies recognized more antigens in the microfilarial stage than in the adult worms. It has also been found that IgE titers were much higher in patients with microscopic dual infection with *Mansonella perstans* and in the group of patients with occult loiasis plus *M. perstans* microfilaremia, compared to individuals with either microfilaraemic *L. loa* or *Mansonella* single infections (62).

Residents from areas of endemicity have been generally noted to have asymptomatic microfilaremia (90% of residents) and occasional episodic Calabar swellings (16% of residents), moderate eosinophilia, and variable antibody levels. Infections in nonresidents were more often symptomatic. Due to increased international travel, the diagnosis of loiasis must be considered in patients who have visited these areas and have a history of eosinophilia, migratory angioedema, and urticarial vasculitis (63). Approximately 10% are microfilaremic and have frequently recurring Calabar swellings, hypereosinophilia of over 70%, increased levels of IgE, and very high antibody titers (64). However, complications such as renal disease, encephalopathy, and cardiomyopathy have been found (64).

The most serious complications include meningoencephalitis, occurring in patients with large numbers of circulating microfilariae who have undergone treatment with DEC; immune complex glomerulonephritis; and endomyocardial fibrosis.

Diagnosis

Since the microfilariae of *L. loa* do not appear in the blood until years after the adult worms or the host reaction to infection is noted, the diagnosis is frequently made on the basis of clinical history (Calabar swellings, worm migrations through the eye, eosinophilia, and residence in an area of endemicity). Microfilariae can be detected in the blood by using methods similar to those used to detect *W. bancrofti* or *B. malayi*. Microfilariae are shed by the females on a random schedule; therefore, multiple samples over a period of days may have to be tested. Individuals not from the area of endemicity usually are amicrofilaremic. The microfilariae exhibit diurnal periodicity; therefore, blood samples should be collected during daytime, preferably between 10 a.m. and 3 p.m. The sheathed microfilariae must be differentiated from those of *W. bancrofti* and *B. malayi* (Fig. 15.4, 15.5, 15.13, 15.14, and 15.15; Table 15.1). Demonstration of the sheathed microfilariae with nuclei extending to the tip of the tail is sufficient for the diagnosis (Fig. 15.4 and 15.13). The infection can also be diagnosed by detection or removal of adults from the eye (Algorithm 15.2, Fig. 15.14).

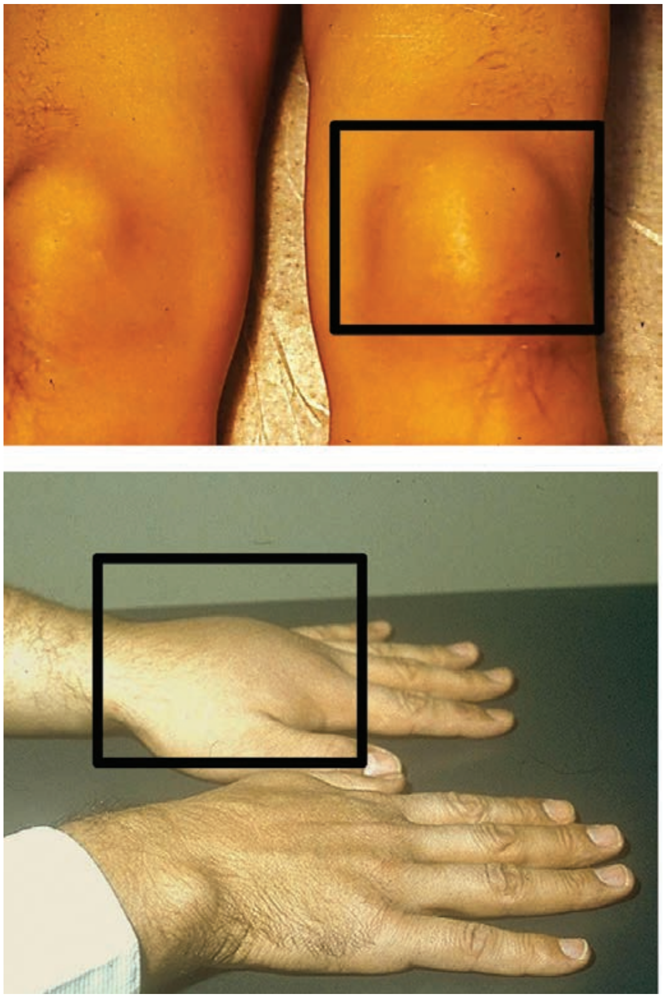
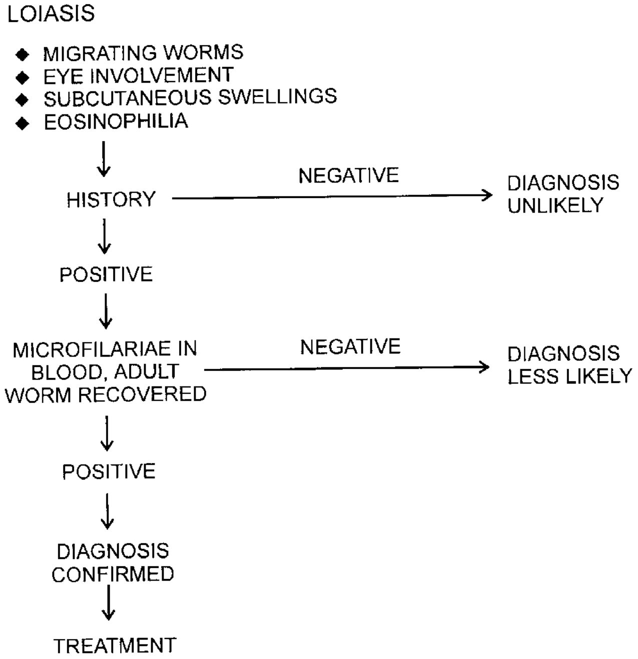


Figure 15.15 *Loa loa.* (Upper) Calabar swelling; note the one enlarged knee (see square). (Lower) Calabar swelling on hand (see square). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch15.f15

Occasionally, in asymptomatic individuals, microfilariae can be recovered from peripheral blood films, urine, sputum, cerebrospinal fluid, cervicovaginal smears, blood vessel biopsy specimens, or autopsy specimens (56). Although Giemsa stain does not reveal the sheath, Delafield's hematoxylin may do so and is recommended. Biopsy specimens of Calabar swellings prior to therapy rarely demonstrate the presence of adult worms.

Treatment

Surgical removal of the adult worms as they are migrating across the bridge of the nose or through the conjunctiva



Algorithm 15.2 Loiasis.
doi:10.1128/9781555819002.ch15.Alg15.2

is relatively simple (Fig. 15.14). DEC is an effective treatment; however, in patients with a heavy microfilaremia, anti-inflammatory drugs may also have to be administered to reduce severe side effects. The most serious sequelae are neurologic complications resembling the encephalitis that is commonly associated with a high microfilaremia. If neurologic disorders develop, it is mandatory that therapy with DEC be stopped. Some individuals may require multiple courses of therapy to be clinically cured. Although DEC is curative in most individuals, relapses can occur within the first 12 months and up to 8 years after treatment (65). In areas of endemic infection, DEC at a prophylactic dose of 300 mg weekly is effective in preventing loiasis in long-term visitors (66). The occurrence of serious adverse effects following DEC treatment of onchocerciasis in areas where *L. loa* is endemic has been increasingly reported over the past decade. These include a severely disabling, and potentially fatal, encephalopathy, which appears to correlate with a high load of *L. loa* microfilariae (>30,000/ml).

Both mebendazole and ivermectin have been used to treat loiasis. The use of mebendazole is not promising, whereas ivermectin is effective in reducing microfilaremia (66). Albendazole, a benzimidazole derivative related to mebendazole, is effective in reducing microfilaremia, and there is a lower risk of encephalopathy; however, repeated courses of therapy may be required (65, 66). Individuals treated with albendazole have a significant reduction in the levels of eosinophils, antifilarial IgG, and IgG4. Although there were no cures, it is possible that albendazole directly affects the adult stages.

Epidemiology and Prevention

Loiasis is endemic to the Central and West African rain forests and may infect as many as 13 million people. Disease distribution depends on the vectors, which breed in wet mud at the side of streams under the rain forest canopy. Flies appear to be attracted by the movement of people or vehicles, as well as rising smoke. An excellent example of vector-host interactive sites would include rubber plantations with a dense high canopy and human workers. The *Chrysops* flies are more common in the rainy season, and exposure differences result in higher infection rates in adults rather than children. Although vector control is one means of prevention, the inaccessibility of breeding sites has proven to be a problem. Clearing of growth around houses and use of screens and protective clothing have all been helpful.

In general, control of loiasis also depends to a great degree on chemoprophylaxis with DEC. Although other therapeutic approaches have been tried, including mebendazole and ivermectin, problems remain with both options.

Mansonella ozzardi

Mansonella ozzardi infections are confined to the New World in Central and South America and the Caribbean islands. Areas of endemic infection in South America include northern Argentina, Bolivia, Brazil, Colombia, Ecuador, and Peru. The infection was first noted by Ozzard in an Indian in South America and was described by Manson. Humans are the only known natural definitive hosts, and prevalence rates may reach 70% in certain areas.

Life Cycle and Morphology

The adult male and female worms live in the subcutaneous and connective tissues, and the microfilariae are found in the blood, where they can be ingested by *Culicoides* spp. (midges) or *Simulium* spp. (blackflies), the insect vectors (Table 15.2). The microfilariae are nonperiodic and unsheathed, with body nuclei that do not extend to the tip of the tail, and are 173 to 240 μm long (Fig. 15.4, 15.5, and 15.16). Microfilariae of *M. ozzardi* resemble those of *M. perstans*; however, the tail of *M. ozzardi* tends to be pointed and slightly flexed and has a longer caudal space (Fig. 15.16) (56). Apparently, humans are the only recognized natural definitive hosts.

Clinical Disease

There is no definitive information available regarding the incubation period in humans. The infections generally produce no symptoms (West Indies); however, they may

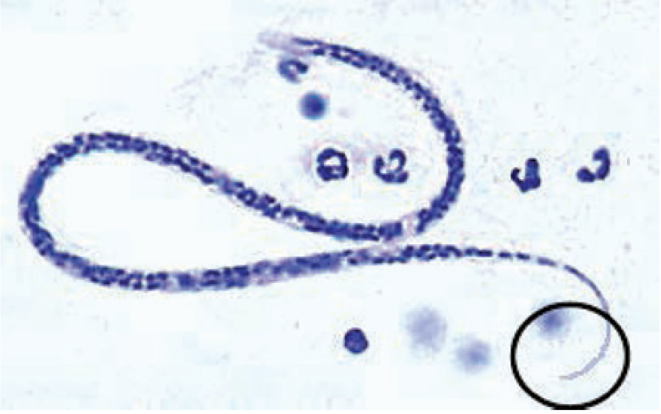


Figure 15.16 *Mansonella ozzardi* microfilaria. Note that the microfilaria is unsheathed and that the tail is pointed and slightly flexed and has a longer caudal space than *M. perstans* seen in Fig. 15.17 (circle) (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch15.f16

cause adenopathy in the lower extremities, knee and ankle pain, pruritus, urticaria, eosinophilia, and fever (Amazon region). Although the worms are generally found in the thoracic and peritoneal cavities and possibly the lymphatics, there is generally no inflammation. Microfilariae were detected in pericardial and pleural fluid from a patient with adenocarcinoma (67). It might be possible to find microfilariae throughout the body of an immunocompromised patient. A cross-sectional survey in the Chaco region of Bolivia showed that 26% (77 of 296) and 0.7% (2 of 298) of the rural populations of two village areas harbored *M. ozzardi* microfilariae. No sex differences were noted, and the lowest prevalence was in 0- to 14-year-old children. There was a sharp increase in 25- to 34-year-old adults, and the prevalence continued to increase in the older age groups. Microfilaremia also increased with age. No significant association between clinical symptoms (fever, skin rash, pruritus, headache, lymphedema, elephantiasis, and articular pain) and microfilaremia was seen (68). A recent study of corneal lesions in the Amazon indicated a 56/212 (26.4%) positivity rate for microfilaremia, with 22/212 (10%) being positive for keratitis. Some lesions were characterized as coin-shaped, mid-periphery and anterior stromal corneal opacities, 0.5 to 1.0mm in diameter with a central mottled appearance surrounded by an opaque halo, and with a marked boundary between the white opacity and the clear cornea. In all cases there was a normal translucent area between the corneal lesions and the limbus and an absence of corneal neovascularization or other changes. Keratitis was more common among patients positive for microfilaremia, while corneal opacity was more common among patients negative for microfilaria, but neither finding was statistically significant (69).

Diagnosis

Methods similar to those used for *W. bancrofti* and *B. malayi* are used to detect microfilariae in the blood. The microfilariae are nonperiodic and must be differentiated from other blood-borne microfilariae (Fig. 15.4, 15.5, and 15.16, Table 15.1). Since the microfilariae are nonperiodic, blood specimens can be taken at any time during the day.

Treatment

Asymptomatic patients need not be treated. Ivermectin has been successfully used to treat infections (45); however, DEC treatment may not be effective. The overall prognosis is excellent.

Epidemiology and Prevention

The infection is indigenous in the Caribbean islands and in Central and South America. *Culicoides* spp. (midges) are the main vectors except in the Amazon basin area of Brazil and Colombia, where blackflies (*Simulium* spp.) are the vectors. Humans are the only known reservoir hosts. The infection may be prevented by the use of insecticides to prevent fly bites. Because of the lack of public health significance of this infection, control programs have not been designed. Travelers are rarely infected.

Mansonella perstans

Mansonella perstans was previously known as *Dipetalonema perstans* or *Acanthocheilonema perstans*. Manson discovered the microfilariae in a blood smear taken from an African patient, and Daniels described the adult worm collected from a South American Indian. In certain areas, infection rates of more than 50% have been reported.

Life Cycle and Morphology

Adult worms are found in the body cavities (peritoneal and pleural), and their microfilariae are found in the blood, where they are ingested by *Culicoides* spp. (midges), the insect vectors (Table 15.2). The microfilariae are nonperiodic and unsheathed, with body nuclei that extend to the tip of the tail, and are 190 to 200 μm long (Fig. 15.4, 15.5, and 15.17). There is no caudal space seen in microfilariae of *M. perstans*, and the posterior end tends to be blunt.

Clinical Disease

Even though the adult worms inhabit the body cavities, there is minimal literature on the pathology caused by this infection. Live adults appear to produce little or no host reaction; however, eosinophilia is common. Pruritus, abdominal pain, urticaria, and Calabar-like swellings on the arms, shoulders, and face have been described. *M. perstans* filariasis has been found in the bone marrow of a human

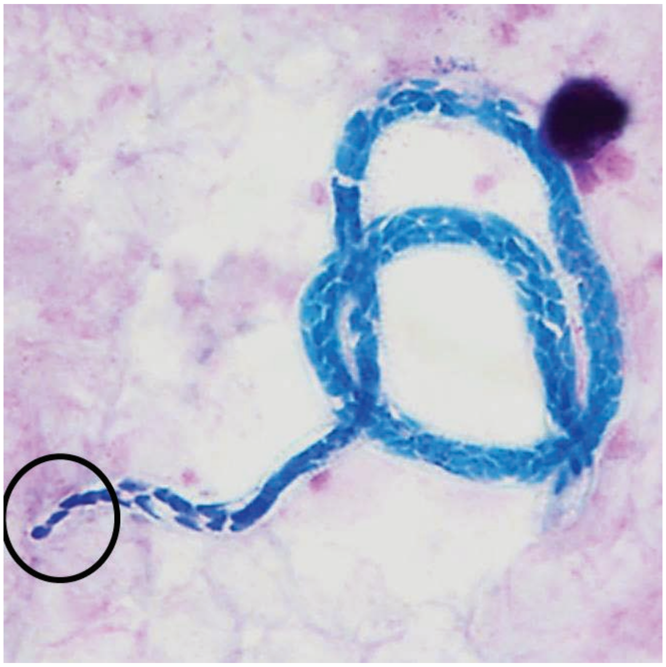


Figure 15.17 *Mansonella perstans* microfilaria. Note that the microfilaria is unsheathed and there is very little tail space (circle) compared with the pointed tail of *M. ozzardi* seen in Fig. 15.16 (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch15.f17

immunodeficiency virus (HIV)-infected patient (70). Ocular symptoms include periorbital edema and conjunctival irritation. In Uganda, acute periorbital inflammation is common; this condition is known as bung-eye or bulge-eye (56). These types of changes are also reported from Nigeria and Sudan.

Diagnosis

The methods used to detect microfilariae in the blood are similar to those used to detect *W. bancrofti* and *B. malayi* microfilariae. The microfilariae are nonperiodic and must be differentiated from other blood-borne microfilariae (Fig. 15.4, 15.5, and 15.15; Table 15.1). However, in Uganda it has also been confirmed that there is a weak but significant diurnal periodicity with peak intensity around 8 a.m. (71). The weak pattern of microfilarial periodicity indicates that the sampling time of blood specimens for diagnosis will have only minor effect on diagnostic sensitivity.

Treatment

Reports on the effectiveness of DEC are contradictory; the microfilariae are eliminated, but repeated therapy is required. However, mebendazole has been used effectively. Ivermectin is not effective in treating *M. perstans* infections. Dead worms release substances that are very antigenic and cause a dramatic immune response; the worms are surrounded by inflammatory exudates, and there may be abscesses, granulomas, and scarring. In one study, six different therapeutic protocols were undertaken for the treatment. DEC reduced microfilarial density but seldom eliminated the infection after a single treatment. Mebendazole was more effective than DEC in eliminating the infection, while ivermectin and praziquantel showed no modification of microfilarial concentration. Thiabendazole demonstrated a small but significant activity against the infection, while combination treatments with DEC and mebendazole showed significantly higher activity than did single drugs alone (72). Combination therapy of ivermectin and albendazole also appeared to be slightly more effective than ivermectin alone, but differences were not statisfically significant (73).

Epidemiology and Prevention

The infection occurs in tropical Africa, the West Indies, and along the Atlantic coast in Central and South America. *Culicoides* spp. (midges) are the insect vectors, and humans, gorillas, and monkeys are the known reservoir hosts. The intermediate host (*Culicoides* spp.) breeds in swamps and jungles, and so eradication or control measures are not practical. Insecticides can be used to prevent fly bites.

Mansonella streptocerca

Until recently, *M. streptocerca* was named *Dipetalonema streptocerca*. It was also previously called *Acanthocheilonema* or *Tetrapetalonema*. Microfilariae were first noted in skin snips obtained from a West African native in 1922, and the adult worms were described in the 1970s. Because there is an overlap in geographic ranges, when found in skin snips, microfilariae of *M. streptocerca* may be misidentified as *Onchocerca volvulus*.

Life Cycle and Morphology

Adult female and male worms live in the dermal tissues, and the microfilariae are found in the skin, where they can be ingested by *Culicoides* spp. (midges), the insect vectors (Table 15.2). The microfilariae are unsheathed, with body nuclei that extend to the tip of the tail, and are 180 to 240 μm long (Fig.15.4 and 15.18). When they are immobile, the microfilariae curve the tail to assume a “shepherd’s crook” configuration.

Clinical Disease

Although unconfirmed, the incubation period is suspected to be several months. There are limited reports on the symptoms and pathology associated with *M. streptocerca* infections, and many patients are asymptomatic. Pruritic

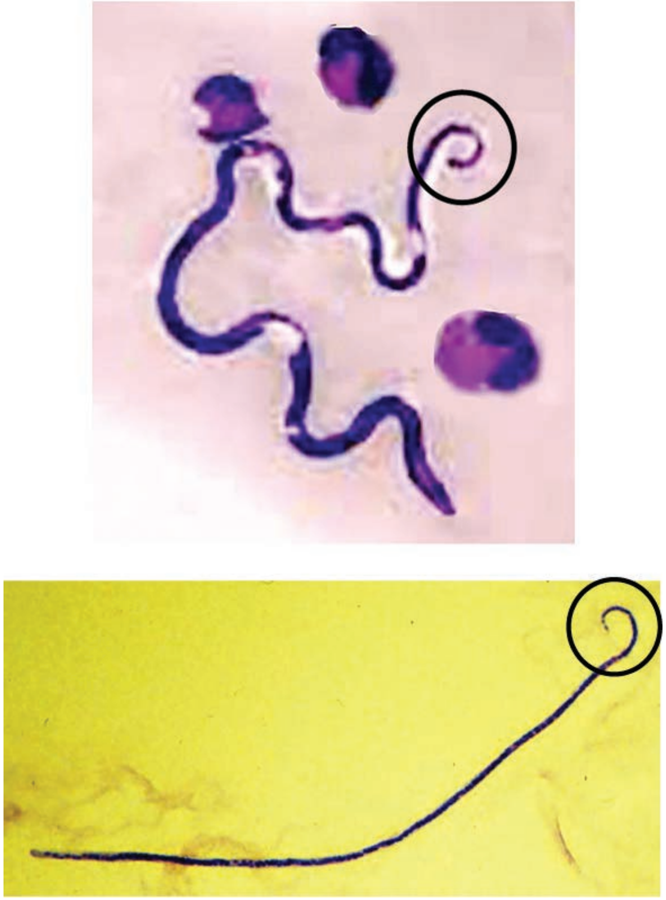


Figure 15.18 *Mansonella streptocerca*. Note the curved tail in both images (circles) (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch15.f18

dermatitis with hypopigmented macules on the skin and lymphadenopathy have been common complaints. Many patients have inguinal adenopathy. In areas where leprosy is endemic, the hypopigmented dermal lesions of streptocerciasis are often misdiagnosed as leprosy; however, no sensory changes are seen, in contrast to leprosy. It is recommended that these lesions be examined histologically for confirmation of either filariasis, leprosy, or both.

Diagnosis

Any patient from the area of endemicity who presents with hypopigmented macules and pruritus should be suspected of having the infection. However, this appearance can be confused with leprosy, and some patients are misdiagnosed and inappropriately treated for leprosy for long periods. The diagnosis is made by the detection of microfilariae from skin snips taken over the scapula and examined as wet mounts. Orihel (74) noted that the microfilarial tails are split rather than blunt, as they were previously described. These microfilariae must be differentiated from *O. volvulus*, which also can be detected in the skin (Fig. 15.4; Table 15.1). The length of *M. streptocerca* is two-thirds that of *O. volvulus*; the microfilariae of *M. streptocerca* are also thinner than *O. volvulus*, and the posterior end of *M. streptocerca* is bent like a shepherd's crook (Fig. 15.18).

Microfilaria can be found primarily in the upper third of the dermis in the dermal collagen. Changes in the skin include sclerosis of dermal papillae, edema, incontinence of melanin, fibrosis, and cellular infiltrates around blood vessels, and the lymphatics are frequently dilated. In untreated patients, adult worms are occasionally found in the dermis; they do not tend to cause an immune response when alive.

Treatment

The infection responds to treatment with DEC; however, a complaint from most patients is the intense pruritus that develops. The long-term effect of a single oral ivermectin dose of 150 µg/kg of body weight on *M. streptocerca* was studied in western Uganda. The results indicate that ivermectin is highly effective against *M. streptocerca* and that a single dose leads to a sustained suppression of microfilariae in the skin. In Africa, ivermectin is used for mass treatment to control *O. volvulus* and *W. bancrofti*. Since these filarial infections are often coendemic with *M. streptocerca*, the treated population may receive suppression benefits for *M. streptocerca* microfilaria as well.

Epidemiology and Prevention

The infection has been found in western and central sub-Saharan Africa, as far east as western Uganda, and as far south as northern Angola. The incidence may reach 90% in certain areas of dense forests of equatorial Congo. Both monkeys and humans harbor the infection; however, there is speculation that the species infecting monkeys may differ from those infecting humans. Biting midges (*Culicoides* spp.) are the insect vectors. There are no control programs aimed specifically at *M. streptocerca* or *Culicoides* spp. Insecticides can be used to prevent fly bites.

Onchocerca volvulus

Onchocerca volvulus microfilariae were first found by O'Neil in 1875, and the adult worms were detected by Leuckart in 1893 in nodules removed from the scalp and chest of a West African native. Onchocerciasis, which is also known as river blindness, is a major public health problem because the infection is a leading cause of blindness in the world and may also cause significant disfigurement of the skin. Approximately 37 million people in 38

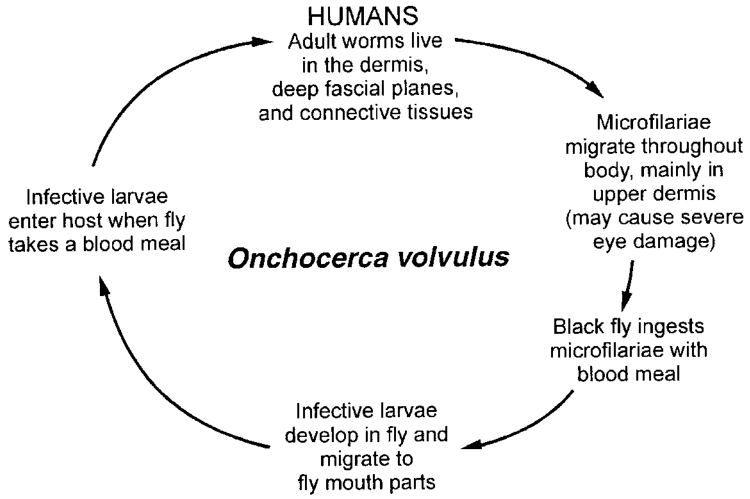

HUMANS
Adult worms live in the dermis, deep fascial planes, and connective tissues
Microfilariae migrate throughout body, mainly in upper dermis (may cause severe eye damage)
Black fly ingests microfilariae with blood meal
Infective larvae develop in fly and migrate to fly mouth parts
Infective larvae enter host when fly takes a blood meal
Onchocerca volvulus

Figure 15.19 Life cycle of *Onchocerca volvulus*. doi:10.1128/9781555819002.ch15.f19

countries in sub-Saharan Africa and South America are infected with *O. volvulus*; of these, 1 million are blind or have severe visual impairment (75, 76). Unfortunately, these figures are probably underestimates, with approximately 123 million at risk for infection.

Life Cycle and Morphology

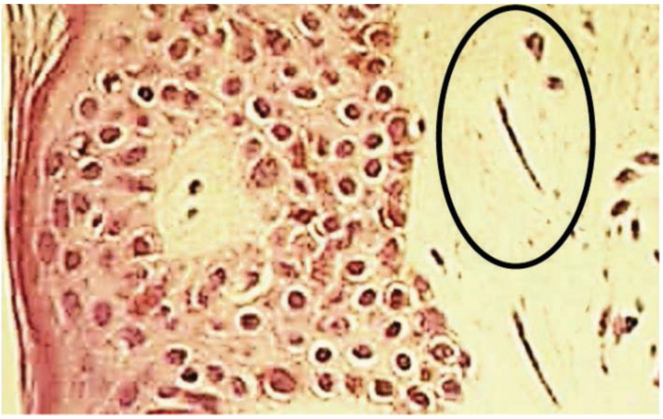
O. volvulus L3 larvae are transmitted by blackflies and develop into adults that are found in subcutaneous nodules (Table 15.2). Adult female and male worms lie within fibrous tissue capsules in the dermis and subcutaneous tissues. The female adult is very long and thin, measuring up to 50 cm by 0.3 mm, while the adult male is 2.5 to 5 cm by 125 to 200 µm. Gravid females produce from 1,000 to 3,000 microfilariae per day, which invade the subcutaneous tissues, skin, and eyes. The infection is transmitted by a species of *Simulium* (blackfly or buffalo gnat). Apparently the blackflies are pool feeders, and their saliva probably contains anticoagulants as well as attractants for the skin-dwelling microfilariae (77). After being ingested, the microfilariae migrate to the flight muscles, become infective in about 6 to 12 days, and migrate to the proboscis, where they are transmitted when the next blood meal is taken. Humans are infected when bitten by the infected fly, and larvae are deposited into the bite site (Fig. 15.19). Microfilariae are normally found in the dermis and rarely in the blood, sputum, or urine. They are unsheathed, with body nuclei that do not extend to the tail tip, and are 221 to 360 µm long (Fig. 15.4, 15.20). The infective larvae undergo two molts during the next 12 months prior to becoming mature adult worms. The gravid females produce thousands of microfilariae daily beginning about 12 to 15 months after infection. The mean reproductive life span of the adult worm is up to 15 years, with maximum reproduction during the first 5 years of patency; the microfilariae may live for up to 15 years (56).

Clinical Disease

The prepatent period in humans is 3 to 15 months. Light infections may produce no symptoms, and worms may be free in the tissue; however, in heavy infections, major disease manifestations include dermatitis, onchocercomas (subcutaneous nodules containing adult worms), lymphadenitis, and blindness. Even though there is an inflammatory reaction that causes the formation of a fibrotic capsule (onchocercoma) around the adult, the main pathology appears to be directed against the microfilariae (Fig. 15.21).

Onchocercomas. Light to moderate infection may produce an itchy rash, while heavy infections (up to 200 million microfilariae) may lead to severe sequelae. Heavy infections are the result of continued exposure to infective larvae over a period of many years. The onchocercoma is a firm,

Figure 15.20 *Onchocerca volvulus* microfilariae in the skin (oval) (cross section). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch15.f20



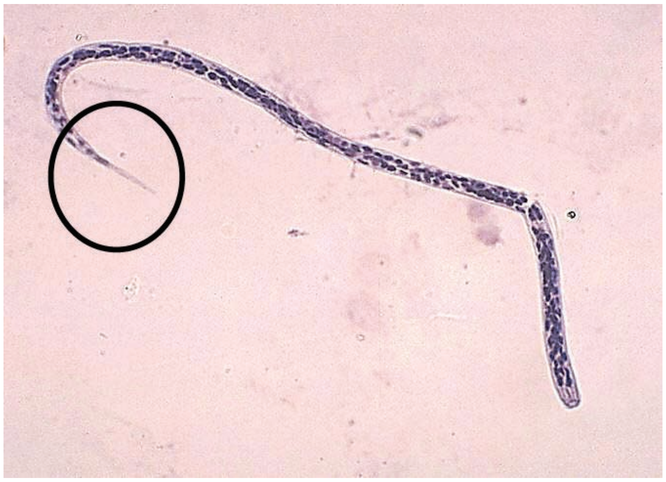


Figure 15.21 *Onchocerca volvulus* microfilariae; note the tail space (circle) (courtesy of the CDC Public Health Image Library).
doi:10.1128/9781555819002.ch15.f21

round, and nontender subcutaneous nodule that contains the adult worms. In Africa, the nodules are commonly found on the trunk (particularly the hip area) or limbs, while in Mexico and Guatemala, they are frequently found on the scalp (Fig. 15.22 and 15.23).

Skin Changes. Some individuals with onchocerciasis have clinically normal skin, whereas others have pruritus and disfiguring skin lesions. The pruritus may be so severe as to cause sleeplessness, fatigue, and weakness, symptoms that may affect the individual's ability to work and interact socially. Scratching often leads to ulcers, bleeding, and secondary bacterial and/or fungal infections. The skin may be painful, hot, and edematous. This inflammatory condition may recur numerous times, eventually resulting in permanent thickening of the skin. Acute attacks of onchodermatitis have resulted in a purplish skin discoloration on the trunk and upper limbs known as mal morado in Central America. Chronically infected skin loses its elasticity and becomes hypertrophic or thickened. As a result of atrophy and loss of skin elasticity, patients may develop premature exaggerated wrinkling of the skin (Fig. 15.24). In Central America, these skin changes are frequently seen as noticeable thickening of the earlobes and thickening of facial skin to mimic leonine facies (seen in lepromatous leprosy). In Africa, the same skin changes occurring in the hip region, usually around the inguinal lymph nodes, produce a condition known as hanging groin, which may predispose infected individuals to inguinal and femoral hernias. Lymphadenopathy in the inguinal and femoral areas is common among Africans (Fig. 15.25).

Skin may become hypo- or hyperpigmented. In Africa, patients may exhibit localized areas of spotty depigmentation surrounded by slightly hyperpigmented zones occurring mainly on the shins; this condition is known as leopard skin (Fig. 15.26). These white areas have an absence of melanin, and the prevalence of skin depigmentation has been proposed as a marker for endemic onchocerciasis.

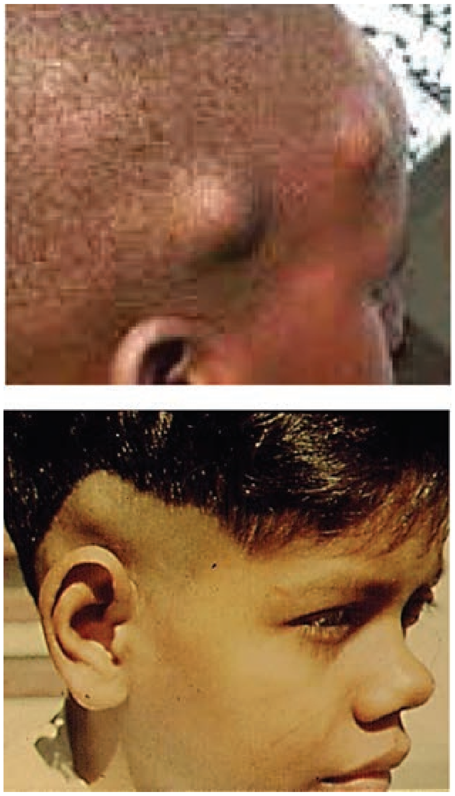


Figure 15.22 Onchocerciasis. The nodules on both patients' heads contain adult worms of *Onchocerca volvulus*. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch15.f22

Sowda is a chronic hyperreactive form of onchocerciasis occurring in a subset of infected individuals, usually in Arabia, Ecuador, Guatemala, Sudan, and West Africa. It is characterized by a severe papular dermatitis usually localized to one limb, typically a leg but may be seen elsewhere, with darkening of the skin (Fig. 15.27). The number of microfilariae in the skin is extremely small, and there is extensive follicular hyperplasia of the regional lymph nodes. Individuals with sowda have significantly higher antibody titers, including IgE and antigen-specific IgG, than do individuals with generalized onchocerciasis (78). These skin lesions improve after therapy. It is possible that this form of onchocerciasis is linked to the formation of autoantibodies to defensins and the hyperimmune response to the parasite.

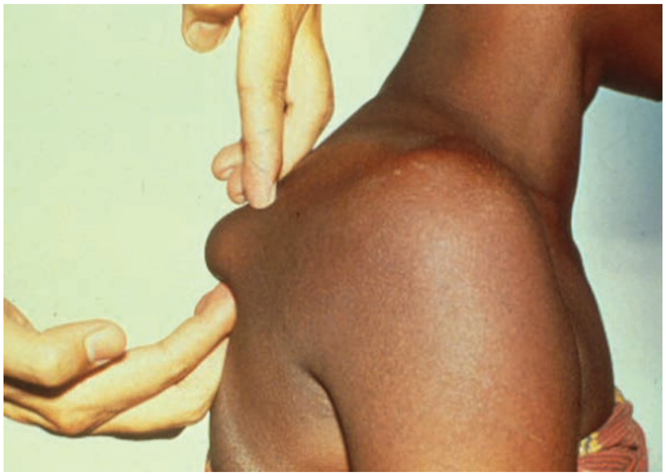


Figure 15.23 Onchocerciasis. Nodules on the back prior to surgical removal. (Courtesy of Medscape, Aileen M. Marty.) doi:10.1128/9781555819002.ch15.f23

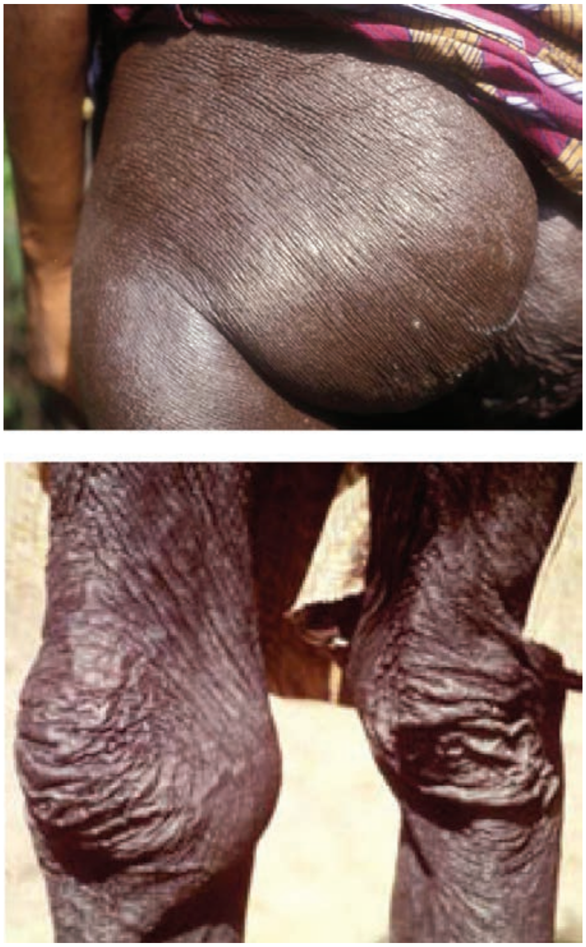


Figure 15.24 Onchocerciasis. Note the exaggerated wrinkling of the skin as a result of atrophy and loss of skin elasticity. (Upper, courtesy of Clare Gilbert, International Centre for Eye Health, London School of Hygiene and Tropical Medicine [www.iceh.org.uk]. Lower, courtesy of United Front against Riverblindness [http://www.riverblindness.org/news-room/gallery-onchocerciasis].) doi:10.1128/9781555819002.ch15.f24

Ocular Involvement. A major complication of onchocerciasis has been ocular lesions leading to bilateral blindness. The prevalence of blindness depends on the organism strain and the prevalence of disease within an area, while the degree of eye involvement depends on the duration and severity of infection (Fig. 15.28). Visual problems can include fluffy corneal opacities, sclerosing keratitis, and iridocyclitis leading to glaucoma and cataracts; chorioretinitis and optic atrophy also occur. Eye damage is caused by living and dead microfilariae in the cornea, anterior and posterior chambers, iris, retrolental space, vitreous, choroid, retina, sclera, and optic nerve. Progressive pathology generally develops over time, with resulting blindness seen in adults.

Ocular lesions are a result of the host immune response to the microfilariae. Circulating antibodies to retinal antigens have been found in patients with onchocercal retinopathy (79). Whether these autoantibodies play an appreciable role in ocular pathology is unknown. Chorioretinal changes have been noted in 75% of the population in areas of West Africa where the infection is endemic.

In West Africa, the severity of ocular pathology is based on strain differences as confirmed by DNA sequence determination. Classification of these strains based on sequence differences correlates very well with pathologic differences. PCR testing has been used to map the distribution of the blinding and nonblinding strains in Nigeria (75). Preliminary studies indicated that certain species of blackfly transmitted the different strains; delineation of these transmission zones has become more difficult, and transition zones appear to contain both strains and/or hybrids. Ocular problems such as severe sclerosing keratitis, anterior uveitis, and blindness are more prevalent in the savanna regions of West Africa but much less common in the rain forest areas. Data also support the hypothesis that the pathogenic differences seen in severe and mild strain onchocerciasis may be due to their relative *Wolbachia* bacteria burden and that *Wolbachia* products may play a major role in the pathogenesis of ocular onchocerciasis (80). It has been shown that endosymbiotic *Wolbachia* is essential for the pathogenesis of keratitis, as (i) *O. volvulus* from individuals depleted of *Wolbachia* by antibiotic treatment does not induce corneal inflammation, (ii) related filarial species containing *Wolbachia* induce keratitis, in

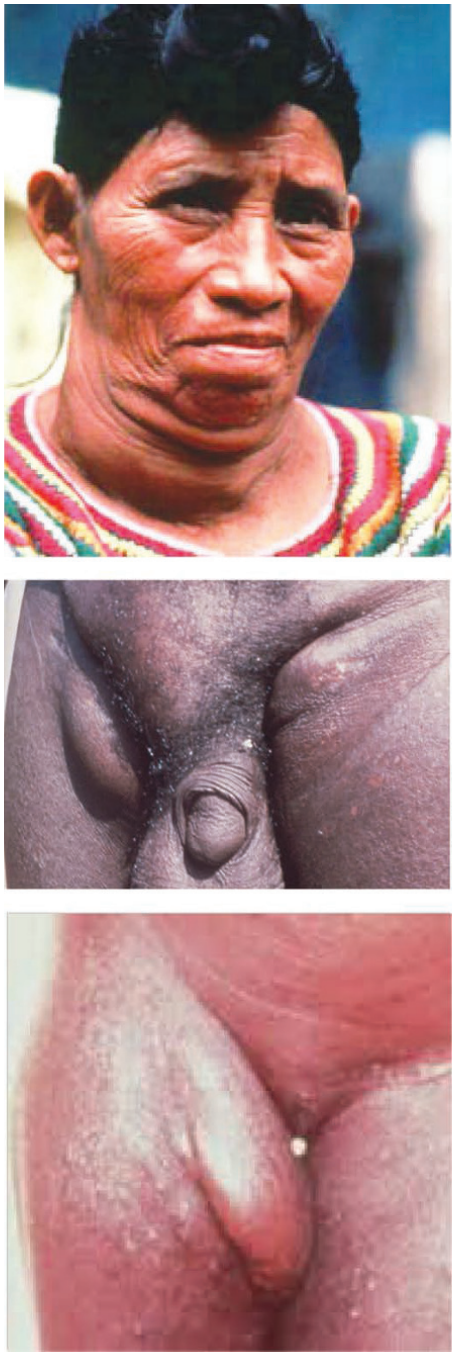


Figure 15.25 Onchocerciasis. (Top) Premature aging seen as a result of skin changes. (Middle and bottom) Hanging groin due to femoral lymphadenitis. (Courtesy of Brooke Army Medical Center teaching file; images in public domain.)
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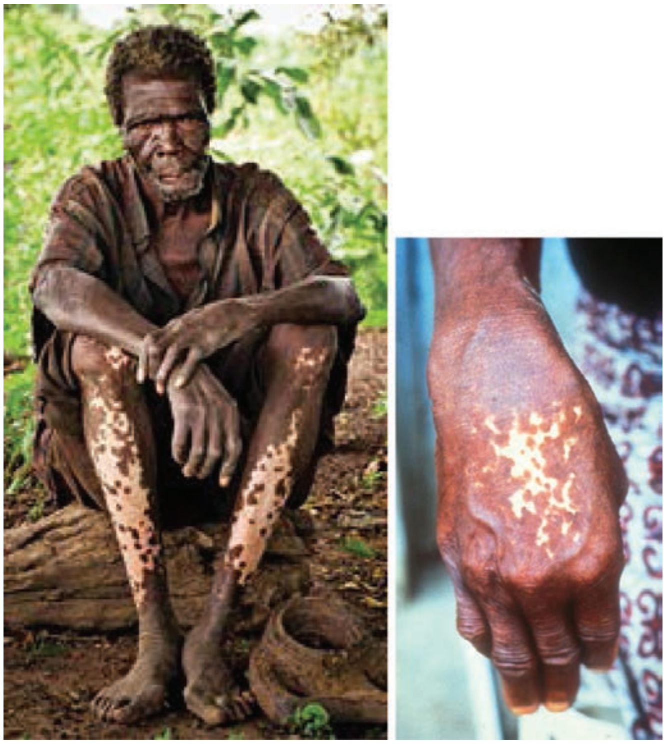

Figure 15.26 Onchocerciasis. Note the localized areas of spotty depigmentation surrounded by slightly hyperpigmented zones occurring mainly on the shins; this condition is known as leopard skin. (Courtesy of the Carter Center.)
doi:10.1128/9781555819002.ch15.f26

contrast to filarial species lacking *Wolbachia*, and (iii) isolated *Wolbachia* bacteria induce neutrophil recruitment to the corneal stroma (81).

Immune System Effects. In individuals with microfilardermia, there is a lack of a cellular immune response; however, there is a Th2 response, which is most rigorous in patients with the sowda form of onchocercal dermatitis. Individuals with chronic onchocerciasis have an abundance of T cells producing IL-4 and IL-5, and they have markedly high levels of antigen-specific IgE and IgG4 antibodies and eosinophilia (82).

Studies suggest a role for mast cells and tissue basophiles in the pathogenesis of onchocerciasis. The IgE bound to the plasma membrane of these mediator-rich cells could contribute to the inflammatory reaction; parasite-soluble components and cuticular proteins are inducers of histamine release. Possible recognition of cuticle internal matrix could occur during molting and after attack by immune responses directed against other targets. If this is true, histamine-containing cells could participate in the body's response to the parasite. The chronic inflammatory reac-

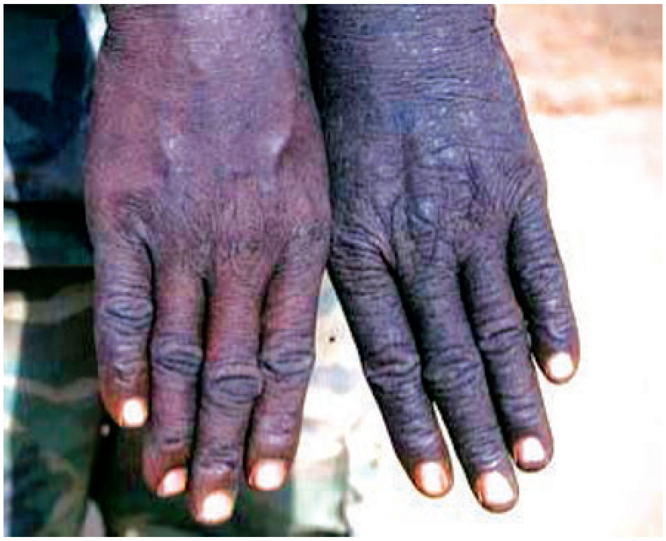

Figure 15.27 Onchocerciasis. Man demonstrating the difference in color and appearance of his hands due to sowda. One hand is significantly darker and thicker. (Courtesy of WHO/TDR/Crump.) doi:10.1128/9781555819002.ch15.f27

tion seen in onchocerciasis may be mediated by mechanisms involving IgE. Product release from basophiles or mast cells acting on eosinophils could be very relevant to the outcome of the infection (83). It also appears that the eosinophil chemokine eotaxin plays a role in recruitment of eosinophils to the dermis in early-stage onchocercal skin disease (84).

In Uganda, as well as in other areas of Africa, *O. volvulus* and HIV are two immunocompromising infectious agents of major health concern. Cellular immune responses in patients coinfected with these two agents suggest an HIV-related lack of specific reactivity to *O. volvulus* antigen and impairment of IL-4 and IL-5 production in addition to the lack of an IFN-γ response to antigenic stimulation (85).

Prostaglandin E(2) and transforming growth factor-β (TGF-β) are two immunoregulative mediators found in macrophages and plasma cells. TGF-β was seen more often in infiltrating macrophages in patients with a hyporeactive onchocerciasis and less in patients with the hyperreactive form. Also, the presence of E(2) and TGF-β in host cells infiltrating the onchocercoma and dermis may suggest containment of inflammatory responses that could enhance the survival of the parasite (86).

Neurologic System Effects. In 2010, reports indicated a recent increase and geographic clustering of an illness resulting in head nodding and seizures. This nodding syndrome has been described as an unexplained neurologic condition characterized by episodes of repetitive dropping forward of the head and often accompanied by convulsions or staring spells, all considered forms of epilepsy. The condition predominantly affects children aged 5 to 15 years and has been reported in South Sudan from the states of Western and Central Equatoria and in northern Uganda and southern Tanzania. Although onchocerciasis was more prevalent among case patients and has been suspected as the causative agent, whether infection preceded or followed nodding syndrome onset is unknown (87, 88). There is also evidence to support the hypothesis that the intensity of infection is probably involved in the etiology of epilepsy (89). There is no cure, and treatment is symptomatic with common anticonvulsants to improve the quality of life.

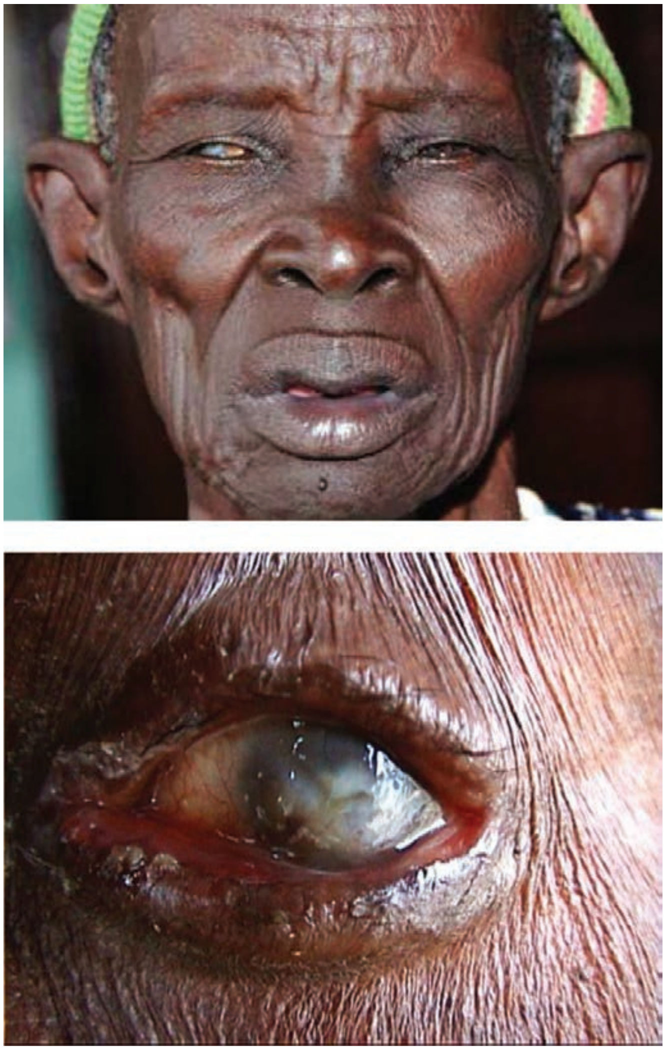

Figure 15.28 Blindness caused by onchocerciasis. (Upper, courtesy of Christian Blind Mission UK; lower, courtesy of WHO/TDR.) doi:10.1128/9781555819002.ch15.f28

Diagnosis

In areas where the infection is endemic, the clinical diagnosis of onchocerciasis is not difficult when individuals present with typical features such as hanging groin, leopard skin, skin atrophy, or subcutaneous nodules. However, skin lesions must be differentiated from scabies, insect bites, streptocerciasis, contact dermatitis, hypersensitivity reactions, traumatic or inflammatory depigmentation, tuberculoid leprosy, dermatomycoses, and treponematoses.

Microfilariae are commonly detected in the skin and must be differentiated from *M. streptocerca* microfilariae, which are also found in the skin (Fig. 15.4, 15.18; Table 15.1). Multiple skin snips are recommended, with the sensitivity of six snips per person being estimated at 91.6% (90) (Fig. 15.29). Infrequently, microfilariae are found in the urine, blood, or sputum, particularly after initiation of DEC treatment. Skin snips should be examined 3 to 6 months after therapy to determine whether the treatment was successful (Algorithm 15.3). PCR has been used successfully with skin snips to detect infections. The PCR method is significantly more sensitive than routine microscopic methods in detecting microfilariae in skin snips and would be more reliable in detecting active infections than are serologic assays (91). Microscopic examination of skin snips cannot detect prepatent or low-level infections.The diagnosis can also be confirmed by finding onchocercomas and adult worms within fibrotic tissues. Ultrasonography can distinguish onchocercomas from lymph nodes, lipomas, fibromas, and a foreign-body granuloma. A typical nodule appears as a central homogeneous area containing some dense particles with a lateral acoustic shadow.

In addition, slit-lamp examination of the eye to detect and visualize microfilariae in the anterior chamber or retroillumination to detect corneal microfilariae can be performed. Patients are instructed to place their heads between their knees for about 10 min. Once they sit up, the microfilariae become concentrated behind the central cornea and are easier to see and count.

The Mazzotti test can be used when results with skin snips are negative; however, there may be severe reactions in patients with heavy microfilarial burdens. A single oral dose of 25 to 50 mg of DEC is given; individuals often develop intense pruritus within a few hours. The itching can be severe and may be accompanied by erythema, edema, and papules; it usually subsides within a few days. Topical application of DEC in a patch test eliminates some of these problems; the appearance of a localized inflammatory reaction at the site of the patch indicates a positive test. The Mazzotti test should be limited to patients with light infections who have no microfilariae in the eyes.

The use of recombinant antigens for serologic diagnosis can help overcome the problems associated with poor sensitivity and specificity, particularly in areas where other human filarial infections overlap with those caused by *O. volvulus* (92, 93). A cocktail of recombinant *O. volvulus* antigens for serologic diagnosis with the potential to predict the endemicity of onchocerciasis infection has been developed (94). When a sentinel population of 5- to 15-year-old children was used to compare communities, the serologic index was sufficiently sensitive to measure the changes in endemicity that would be required for control programs. However, serologic testing cannot distinguish between current and past infection and tends to be more important in monitoring specific groups.

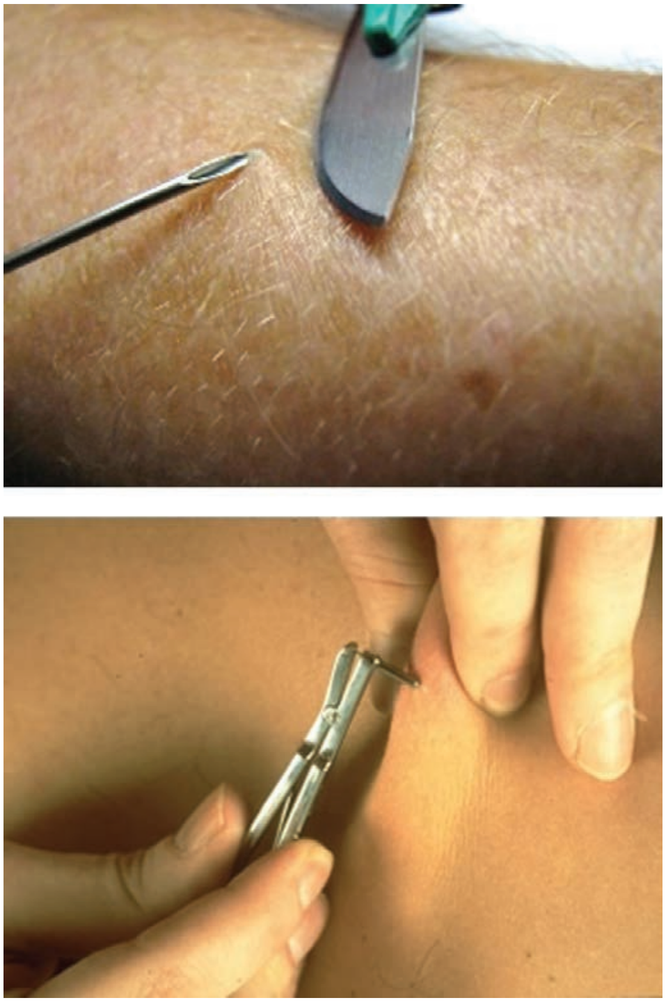

Figure 15.29 Onchocerciasis. (Upper) A physician takes a skin sample from a patient for a skin snip biopsy by elevating a piece of skin with a needle and shaving it off with a scalpel (there should be little to no blood). (Lower) A physician takes a skin sample from a patient for a skin snip biopsy using a sclerocorneal biopsy punch. (Images courtesy of Thomas B. Nutman, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health.)
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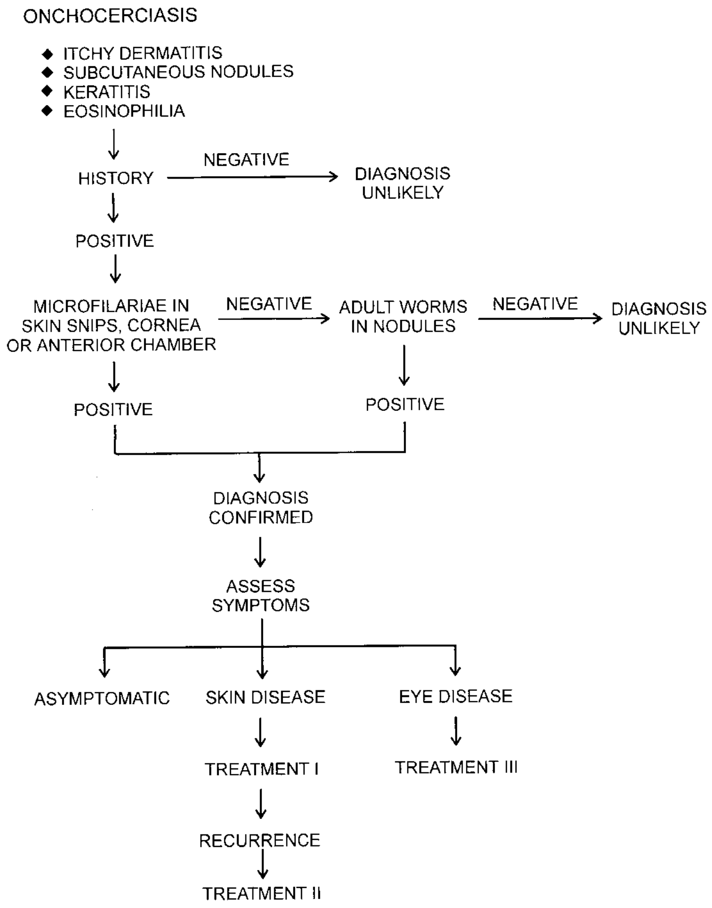
ONCHOCERCIASIS
◆ ITCHY DERMATITIS
◆ SUBCUTANEOUS NODULES
◆ KERATITIS
◆ EOSINOPHILIA
HISTORY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
MICROFILARIAE IN SKIN SNIPS, CORNEA OR ANTERIOR CHAMBER
NEGATIVE
ADULT WORMS IN NODULES
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
POSITIVE
DIAGNOSIS CONFIRMED
ASSESS SYMPTOMS
ASYMPTOMATIC
SKIN DISEASE
EYE DISEASE
TREATMENT I
TREATMENT III
RECURRENCE
TREATMENT II

Algorithm 15.3 Onchocerciasis.
doi:10.1128/9781555819002.ch15.Alg15.3

Treatment

Surgery. Surgical removal of detectable nodules, especially in the head region, is simple and easy to perform (Fig. 15.30). Nodule removal from the head, particularly in children, reduces the risk and severity of ocular complications. Removal of adult worms decreases the exposure of the host to microfilariae, which helps decrease the risk of complications. Because many nodules are nonpalpable or the adults may be freely migrating, the removal of nodules may have little effect on the total microfilarial or worm burden. Removal of enlarged regional lymph glands has been traditional therapy for sowda in Yemen.

Ivermectin. The treatment and control of onchocerciasis rely on a single drug, ivermectin. This is used in mass drug administration either annually or biannually or for individual treatment every 3 to 6 months. It effectively reduces microfilarial loads but is only marginally effective against adult worms. Consequently, sustained delivery for more than 15 to 17 years is required in order to interrupt transmission. Ivermectin markedly reduces microfilaria numbers within 48 h and blocks the release of microfilariae from the uterus of the adult female worm for up to 6 months. Following treatment, microfilariae disappear from the skin and can be detected in large numbers in the regional lymph nodes, where there is a high concentration of eosinophils. Ivermectin is well tolerated, but its side effects include pruritus, skin edema, and arthralgia; however, these effects are not as severe as those noted with DEC treatment. Ivermectin can also cause renal glomerular and tubular disturbances in patients with onchocerciasis; however, these are minor and do not seem to be clinically relevant. Although ivermectin significantly reduces the microfilarial burden in the host, thereby reducing dermatitis, it has no effect on depigmentation and scarring. Ivermectin treatment even in single doses reduces the prevalence of ocular microfilariae, including the number found in the anterior chamber of the eye, resulting in improvement of ocular lesions and visual acuity. When used in multiple doses, ivermectin is not only microfilaricidal but also macrofilaricidal (i.e., it kills adult worms). However, total doses of ivermectin up to 1,600 µg/kg are not significantly more effective than 150 µg/kg in killing the adult worms; more effective drugs against the adult worms are still needed. Ivermectin is contraindicated for pregnant women, children younger than 5 years, children weighing less than 33 lbs (15 kg), and patients with concurrent illness. Even though pruritus was frequent, no ocular or systemic complications were noted.

Unfortunately, there are reports of suboptimal responses to ivermectin based on the examination of repopulation levels of microfilaria 1 year after treatment, worm burdens per nodule, the age structure of adult female worms recovered from nodules, and the reproductive status of adult female worms 90 days after ivermectin treatment (95). In some areas, the data are consistent with an emergence of ivermectin nonresponse or resistance, shown by a loss of the effect of ivermectin on the suppression of parasite reproduction. Evidence includes the following: the microfilaricidal response is normal; there is early skin repopulation with microfilariae; there are high microfilarial recovery rates 1 year posttreatment and many subjects with more than 10 microfilariae per snip; and many live microfilariae are present *in utero* in female worms recovered 90 days after treatment. This phenotypic evidence of emerging ivermectin resistance will lead to a reevaluation of control strategies and a search for the genotypic correlate that would unequivocally confirm the development of resistance.

Drugs against *Wolbachia*. Although drugs against *Wolbachia* have been highly effective compared with existing onchocerciasis drug regimens that provide permanent sterilization and macrofilaricidal activity against adult *O. volvulus*, prolonged treatment for 4 to 6 weeks and contraindications for doxycycline (pregnancy and age <8 years) remain obstacles to widespread use of these regimens in current control approaches. Doxycycline was the first drug against *Wolbachia* used in trials in human onchocerciasis. Treatment resulted in *Wolbachia* depletion of more than 90%, which is an empirical threshold resulting in death of the worms (96). In order to eliminate doxycycline contraindications, the "Anti-Wolbachia" (A-WOL) consortium was formed in 2007 and funded by the Bill & Melinda Gates Foundation to identify new drugs for this purpose (97).

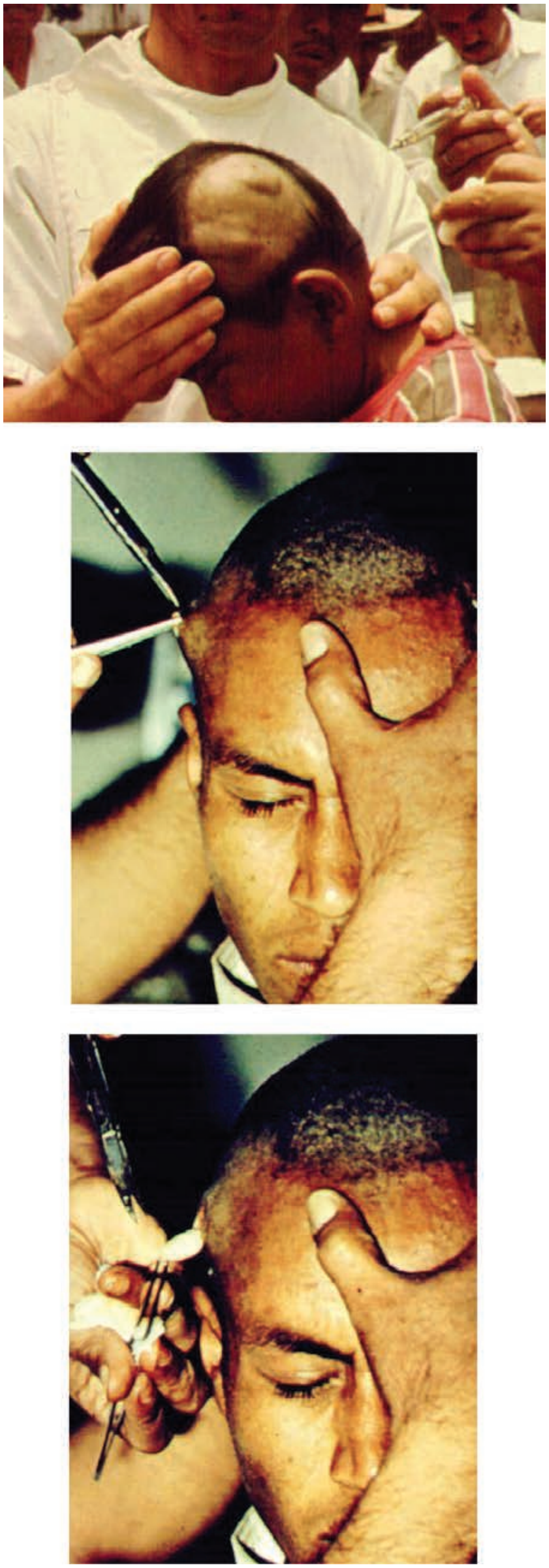

Figure 15.30 Onchocerciasis. (Upper) Injection of anesthetic prior to nodule removal. (Middle) Incision and nodule removal. (Lower) Nodule on the head prior to final removal. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch15.f30

Other drugs. DEC is an effective microfilaricidal drug, but it may precipitate serious dermal and systemic complications as well as ocular damage in heavily infected patients. It has little or no effect on adult worms. The side effects resulting from the death of the microfilariae can be reduced by the use of anti-inflammatory drugs. Another method commonly employed is to gradually increase the DEC doses to therapeutic levels.

Suramin is effective in eliminating adult filarial worms as well as the microfilariae; however, it must be administered systemically and is very nephrotoxic. DEC and suramin in combination have been effective and well tolerated.

Mebendazole and flubendazole (benzimidazole derivatives) are effective microfilaricidal drugs; they are useful alternatives to DEC, and fewer ocular side effects were noted. Mebendazole is poorly absorbed and requires multiple doses. It is teratogenic and embryotoxic in laboratory animals and is contraindicated in pregnancy. Although flubendazole is not teratogenic, a complication is ulceration of the injection site (37).

Epidemiology and Prevention

Onchocerciasis is widely distributed throughout Central Africa, occurs in Saudi Arabia and Yemen, and is found in areas of Brazil, Colombia, Venezuela, Ecuador, Guatemala, and Mexico. An estimated 123 million people are considered at risk for onchocerciasis worldwide. There appear to be no known important animal reservoirs, although natural infections in gorillas have been found in Africa.

The first report of zoonotic *Onchocerca lupi* in the United States (Arizona) has been published (98). The patient was a 22-month-old child who presented with neck pain and stiffness. Magnetic resonance imaging demonstrated an extradural mass extending from C2 through the C4 level with moderate to severe compression of the cord. Examination of a biopsy of the rubbery tumor revealed a gravid female *O. lupi*. The parasite has been reported in dogs and cats in the western United States, and from people in four cases in Europe. Per followup, the patient's symptoms gradually resolved.

Simulium spp. (blackfly or buffalo gnat) are the insect vectors, and they are usually found near rivers and streams. Many species require fast-flowing streams for larval development. River blindness is the common name for onchocerciasis as a result of the frequency of blindness in people living along rivers and streams in areas of endemic infection. In some areas, control programs may be misdirected because of the inability to distinguish animal from human onchocercal larval forms in infected flies. DNA probes to distinguish larval forms could help in targeting the areas where human infections are most frequent. Pool screen PCR for estimating the prevalence of *O. volvulus* infection in the vectors has been used in epidemiologic surveillance for infection (99).

Treatment of streams and rivers with insecticides has successfully reduced or eliminated transmission. A significant problem in vector control has been the development of resistance to larvicides. A solution to this problem is the rotational use of different larvicides during the treatment campaign. Because of the long life span of the adult worms (15 years or more), campaigns must be continued for extended periods.

In areas where application of insecticides would be difficult, surgical removal of nodules has been used as a means of controlling as well as preventing blindness. Chemotherapy as a means of control has not been previously used because of severe side effects from microfilarial death. Because ivermectin can be given orally, the number of doses is limited in comparison with DEC, and compliance is better than with DEC because ivermectin has fewer side effects. Ivermectin therapy could be used as a control measure to reduce transmission in areas of endemicity. The disadvantages of this approach are cost, the fact that ivermectin is not effective against adult worms unless used in multiple doses, and the finding that in areas where *L. loa* also exists, treatment may induce encephalitis in *L. loa*-infected individuals. In communities where ivermectin has been used on a large scale with multiple doses over time, significant reductions in the number of infected flies and the number of infective larvae transmitted have been noted. Significant decreases in the prevalence of onchocerciasis in children have been noted when mass treatment with ivermectin has been carried out in areas of endemic infection. The use of ivermectin in control and eradication programs holds considerable promise. A search for vaccine candidate immunogens is under way.

Dirofilaria Dirofilaria and *Dirofilaria Nochtiella* spp.

Currently, there are two subgenera, *Dirofilaria Dirofilaria* and *Dirofilaria Nochtiella*, based on the presence or absence of external longitudinal cuticular ridges (56). *D. Dirofilaria immitis* is referred to in this volume as *D. immitis*. The others in the *Dirofilaria Nochtiella* species are designated *Dirofilaria* and the species name. A number of these species, such as *D. tenuis*, *D. repens*, *D. ursi*, *D. striata*, and *D. subdermata*, as well as *Brugia* spp., are animal filariae causing subcutaneous or conjunctival nodules in humans (54, 100, 101). Almost all of the worms detected in humans have been nongravid, with the exception of *Brugia* spp. detected in immunodeficient patients and in the right cervical lymph node lymphatic vessel of a normal human and a *Dirofilaria* sp. found in the right lung of a patient with lymphoid leukemia (102). It is possible that the release of microfilariae into the vascular system is suppressed in normal human hosts.

D. immitis, the dog heartworm, causes a common zoonotic filarial infection in dogs in the tropical, subtropical, and warm temperate regions of the world. The infection was prevalent predominantly in the southern coastal and southeastern United States; however, the zone of endemicity has been increasing northward and to the west. Pulmonary lesions are the most common findings in humans infected with *D. immitis*. *D. tenuis* is a parasite of the raccoon within the United States; *D. repens* is found in dogs and cats in Europe, Africa, and Asia and is the cause of human infections in these geographic areas; *D. ursi* infects bears in Canada, the northern United States, and Japan; *D. subdermata* infects porcupines in North America; and *D. striata* infects wild cats such as bobcats, Florida panthers, ocelots, and margays in North and South America (Table 15.3).

Life Cycle and Morphology

Adult female and male worms reside in the right heart of dogs, and microfilariae are found in the blood, where they are ingested by mosquitoes or, in the case of *D. ursi*, *Simulium* blackflies (Fig. 15.31). After biological development of the larvae in the mosquito, dogs and humans may be infected with the infective larvae when the mosquito takes a blood meal. Development to a mature adult takes approximately 180 days in the dog. In humans, the worms do not reach maturity and no microfilariae can be detected.

Adult worms in the *Nochtiella* subgenus live in the subcutaneous tissues of their hosts, although in some cases the worms migrate to the pulmonary vessels. There is often only a single worm; therefore, there is no production of microfilariae.

Clinical Disease

Human dirofilariasis is typically seen as subcutaneous nodules or as lung parenchyma disease. Patients infected with *D. repens* tend to have a subcutaneous lump (face and conjunctiva of the eye and sometimes chest wall, upper arms, thighs, abdominal wall, and male genitalia). Ocular involvement is usually periorbital, orbital, subconjunctival, or subtenon infection. Human *D. immitis* infection is associated with human pulmonary dirofilariasis and is usually asymptomatic. If symptoms are present, they include cough, chest pain, fever, and pleural effusion.

The first reported case in humans was recorded in 1941. Many of the patients were asymptomatic, and pulmonary nodules (coin lesions) were noted on routine X-ray examinations or at autopsy (103). Symptomatic patients exhibited signs and symptoms leading to chest examination, with common findings of chest discomfort, cough, fever, chills, malaise, and occasional hemoptysis. Infections were more frequent in males than in females, and most individuals were 40 to 60 years old.

When the worms lodge in the pulmonary artery branches, they cause an infarct. These lesions are usually on the periphery of the lungs and are sharply defined (coin lesion). There is a central necrotic area surrounded by a granulomatous inflammation and a fibrous wall. Dead or dying worms may be found in the lesion (Fig. 15.32) (104).

In one review, 39 patients were discussed. All were Americans between 8 and 80 years old and included 23 men and 16 women (105). Twenty-two of the patients (56%) were asymptomatic, and the pulmonary nodule was discovered on chest X rays during a routine physical examination. Seventeen patients (44%) presented with general complaints or respiratory symptoms. Only 10% of the patients had a peripheral eosinophilia. Most lesions (76%) were seen in the right lung, with a predilection for the lower lobe. Five patients had multifocal nodules. All patients underwent thoracotomy with excisional lung biopsy or lobectomy. In all cases, histology revealed a histiocyte-rimmed necrotic nodule containing fragments of a partially degenerated *D. immitis* worm, while adjacent lung parenchyma showed morphological features consistent with other pulmonary processes such as extrinsic allergic alveolitis and/or pulmonary vasculitis syndromes. This group of cases highlights the morphologic variation seen in human pulmonary dirofilariasis and emphasizes the need to consider this diagnosis in all cases of necrotizing granulomas of the lung.

Ophthalmic infection with *Dirofilaria* is documented worldwide, including North America, Europe, Australia, Africa, the Middle East, and Asia. Eye involvement may be periorbital, subconjunctival, subtenon, or intraocular. Such lesions are usually associated with moderate to severe inflammation. Most cases with eye infections present with pain in the eye, redness, sometimes blurred vision, and swelling of eyelids. Symptoms usually occur weeks or months after infection with microfilaria (Fig. 15.33).

Diagnosis

Microfilariae cannot be found in the blood or tissues, and serologic results are of little value because of the lack of sensitivity and specificity of this method. Some patients have a moderate eosinophilia. Diagnosis can be confirmed by the identification of worms in surgical or autopsy specimens. Because the immature larvae may be detected in only a few microscopic sections, careful histologic examination is necessary. The cuticle of nematodes contains chitin, which can be stained with nonspecific whiteners such as calcofluor white. *Dirofilaria* larvae stained with calcofluor white can be easily recognized in tissue sections,

Table 15.3 *Dirofilaria* species infecting humans

Species	Length/diameter		Geographic area	Clinical findings	Comments
	Females	Males			
D. immitis	230–310 mm/ 350 μm	120–190 mm/ 300 μm	Throughout the world; tropics to temperate areas	Small pulmonary infarcts usually asymptomatic and appear as "coin lesions"—usually only a single lesion; may have cough, chest pain, fever, pleural effusion	Humans most often infected in United States, Japan, Europe, Australia; no external longitudinal cuticular ridges
D. tenuis	80–130 mm/ 260–360 μm	40–48 mm/ 190–260 μm	Subcutaneous parasite of raccoon in United States	Gravid worm in thigh and abdominal wall (two patients)	External longitudinal cuticular ridges; usually low and rounded
D. repens	100–170 mm/ 460–650 μm	50–70 mm/ 370–450 μm	Subcutaneous parasite of dogs, cats in Europe, Africa, Asia	Can cause pulmonary disease as with *D. immitis*; also recovered from subcutaneous tissue; subcutaneous (normally nodular) and the submucosa (nodular or not); ocular involvement (periorbital, orbital, subconjunctival)	The most commonly affected areas are the head, the thoracic wall, and the upper limbs; most infections seen in Italy; many cases probably not diagnosed, and patients recover spontaneously; external ridges appear beaded
D. ursi	117–224 mm/ 460–700 μm	51–86 mm/ 330–480 μm	Parasitizes bears in Canada, northern United States, and Japan	Usual location of nodules containing the worms is the scalp or a covered part of the upper body where blackflies, the intermediate hosts of *D. ursi*, normally feed; five reports from along U.S.-Canadian border	Case reports indicate worms like either *D. ursi* or *D. subdermata*; distinct longitudinal cuticular ridges; cuticular ridges fewer and farther apart, regularly and widely spaced on the outer surface, usually evident even when the worms are necrotic
D. subdermata	117–185 mm/ 440–660 μm	41–66 mm/ 280–420 μm	Infects porcupines in North America	May see inflamed subcutaneous nodules (painful, erythematous, sometimes migratory)	Very difficult to differentiate female *D. ursi* from *D. subdermata*; now called *D. ursi*-like
D. striata	250–360 mm/ 440–500 μm	80–120 mm/ 350–380 μm	Bobcats in Florida, panthers, ocelots, margays in North and South America	Rare, but produces subcutaneous lesions; also recovered from eye in one child	Ridges may mimic lateral alae

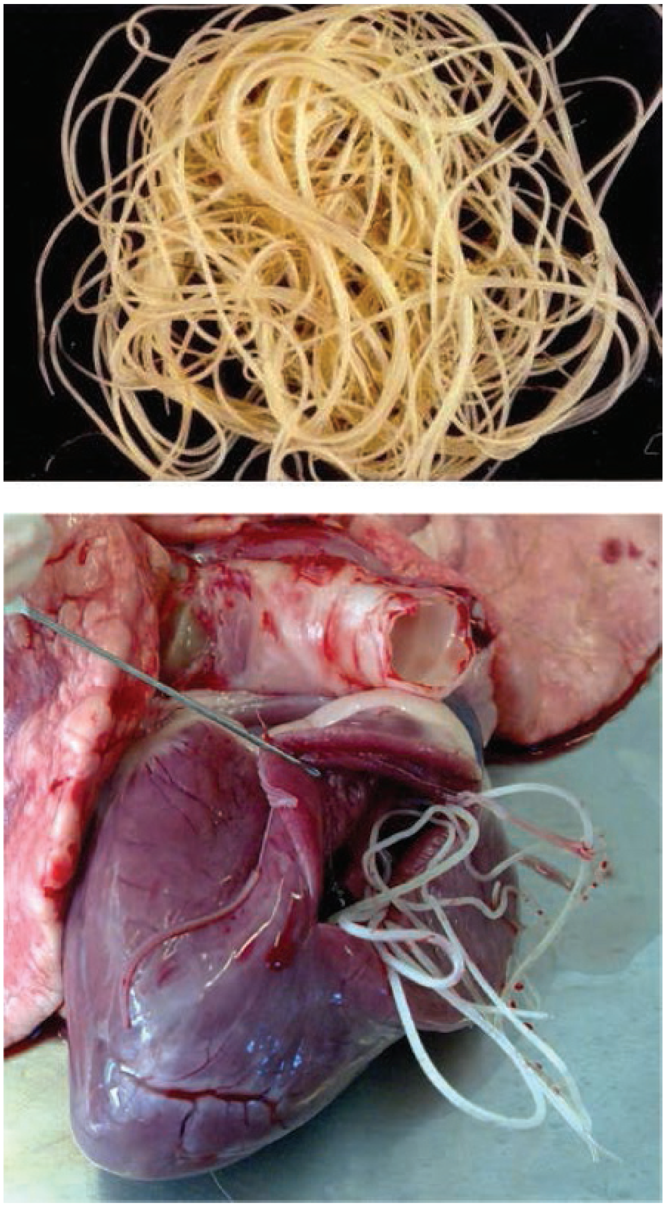

Figure 15.31 *Dirofilaria immitis*. (Upper) Adult worm from dog's heart (courtesy of R. E. Pugh, www.parasite.org.au). (Lower) Adult worms in dog heart (courtesy of Simon F et al, *Clin Microbiol Rev* **25**:507–544, 2012).
doi:10.1128/9781555819002.ch15.f31

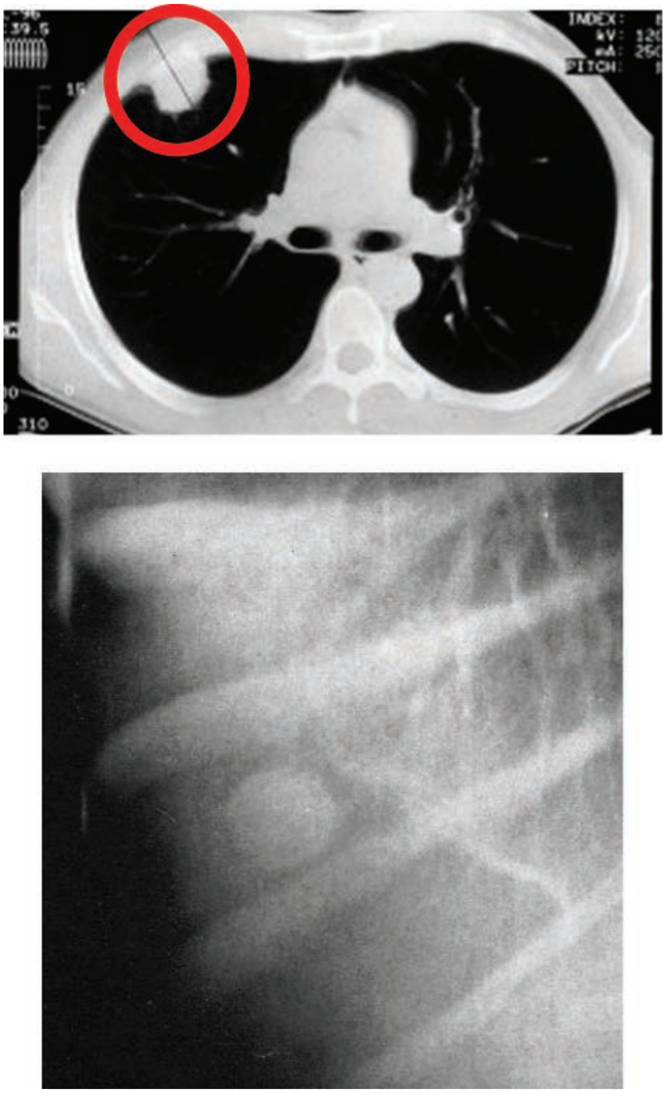

Figure 15.32 Dirofilariasis. (Upper) Computed tomogram image with fine needle in left pulmonary round focus; round focus in right lung periphery (circle) (courtesy of Fueter R, Gebbers J-O, *Schweiz Med Wochenschr* **127**:2014, 1997). (Lower) Chest radiography in pulmonary dirofilariasis. A solitary smooth round nodule in right lower lobe without any clinical symptoms (courtesy of Marty AM, Andersen EM, p 920–927, *in* Doerr W, Seifert G [ed], *Tropical Pathology*, 2nd ed, Springer, Berlin, Germany, 1995). doi:10.1128/9781555819002.ch15.f32

whereas the parasite may be difficult to identify using routine histologic stains.

In a very extensive study of over 90 cases of human infection with *D. repens*, the authors commented on the diagnostic difficulties involved with histologic diagnosis (106). As the worms begin to die within the nodule, the invasion of inflammatory cells begins at the worm's orifices (mouth, vulva, anus, and cloaca) and gradually spreads throughout its body. It is recommended that the nodule be sectioned at different points in order to review morphologic features that may still be recognizable. This review also contains a series of color photographs in which the nematodes are in an excellent state of preservation, while other photographs illustrate the range of disintegration of the worm within the nodule (Fig. 15.34). Dirofilaria are identified by their thick laminated cuticle, broad lateral

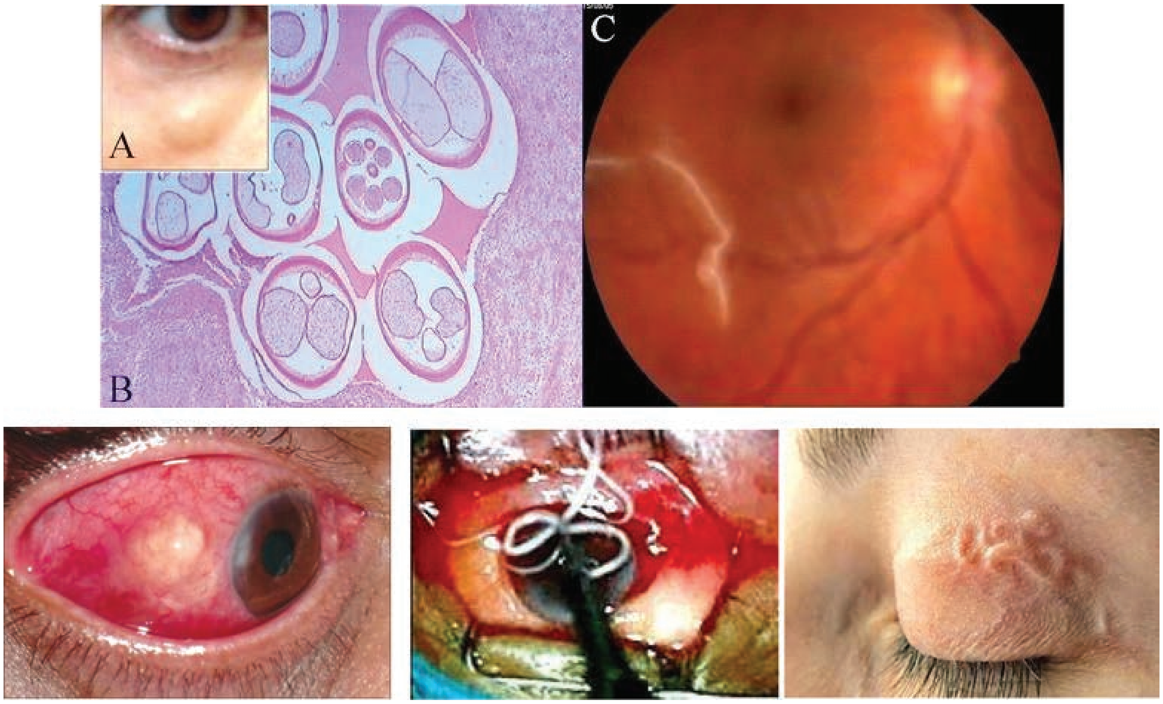

Figure 15.33 Human subcutaneous and ocular dirofilariasis. (Upper) (A) External appearance of a subcutaneous nodule in the ocular region. (B) Histological section of a nodule showing sections of adult *D. repens* worms. (C) Intravitreal location of an adult *D. repens* worm in a human patient. (Panels A and B courtesy of Vladimir Kartashev, University of Rostov Na Donu, Rostov, Russia. Panel C reprinted from Korkhov AP et al, *Med Parazitol [Mosk]* **1**:59, 2009, from Simon F et al, *Clin Microbiol Rev* **25**:507-544, 2012). (Lower, left) Localized conjunctival congestion with a yellowish cream, firm, nonmobile, nontender, scleral nodule (courtesy of Sangit VA, Haldipurkar SS, *Indian J Ophthalmol* **60**:76–77, 2012); (center) removal of *Dirofilaria* adult worm from eye; (right) *Dirofilaria* in eyelid. (Lower center and right, from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch15.f33

ends, and large muscle cells. *D. immitis* can be differentiated from *D. repens* by the absence of ridges.

Human pulmonary dirofilariasis is often a benign and self-limiting condition that does not require immediate treatment. However, it is often confused with other diseases, including tumors, tuberculosis, fungal infections, and benign hamartomas, and invasive surgical procedures are generally required in order to arrive at a definitive diagnosis. Unfortunately, misdiagnosis occasionally leads to inappropriate and harmful therapeutic interventions. For example, this may occur if dirofilariasis infection in the breast were mistaken for breast cancer and chemotherapy were prescribed to treat the cancer.

Treatment

Lesions identified in the lungs are frequently removed when cancer is suspected (57). The correct diagnosis can be confirmed by examination of histologic preparations after surgery. Identification of the worm in biopsy specimens or extraction of the worm from the lesion will confirm the diagnosis. Surgical removal of the worms is the only known treatment. No chemotherapeutic agents are used, since they appear to be ineffective.

Epidemiology and Prevention

Although at least 15 species of mosquitoes can carry the infective forms and the infection is considered an emerging zoonosis, probably fewer than 1,000 cases of human infection have been described. However, it is quite possible that many infections go undiagnosed and the number of cases is larger than documented. Canines, cats, foxes, muskrats, otters, and sea lions are natural hosts. The infection in animals has been noted in the United States, Africa, Mediterranean countries, Southeast Asia, and the Pacific Islands. The prevalence of infection in dogs in the southeastern United States may be as high as 40%.

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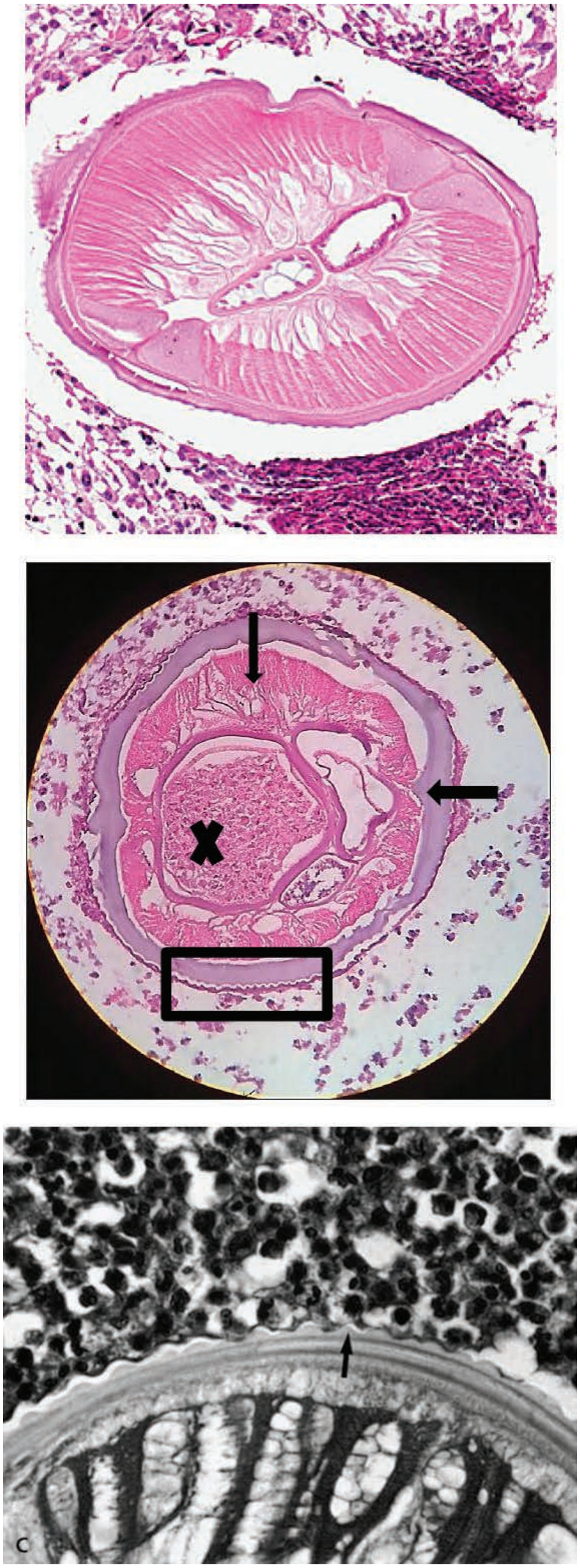

Figure 15.34 Dirofilariasis. (Top) *Dirofilaria* sp. (suspect *D. tenuis*) removed from the eye of a patient (courtesy of the CDC Public Health Image Library). (Middle) Cross section of *Dirofilaria repens* adult worm consisting of thick multilayered cuticle (arrow), transverse striations (arrow), longitudinal wavy ridges (rectangle), and gravid uterus (X) (courtesy of Srinivasamurthy V et al, *Annals Trop Med Pub Hlth* **5**:349-351, 2012). (Bottom) A section from a subcutaneous mass due to *Dirofilaria tenuis* showing the marked eosinophilic reaction; the arrow shows the regularly spaced longitudinal ridges and laminations of the cuticle of the worm (courtesy of Philip Palmer and Maurice Reeder, *The Imaging of Tropical Diseases*, Uniformed Services University of the Health Sciences [distributed by the American College of Radiology, the RSNA, the Radiology Outreach Foundation, and The International Society of Radiology with the kind permission of Springer Publishing, the authors, and the above institutions]). doi:10.1128/9781555819002.ch15.f34

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16

Intestinal Cestodes

Diphyllobothrium latum
Taenia solium
Taenia saginata
Taenia asiatica (Asian *Taenia* or *Taenia saginata asiatica*)
Hymenolepis (*Rodentolepis*) *nana*
Hymenolepis diminuta
Dipylidium caninum

Adult cestodes, or tapeworms, live attached to the mucosa in the small intestine and absorb food from the host intestine. The attachment organ is called the scolex, to which is attached a chain of segments or proglottids called the strobila. Each proglottid contains a male and female reproductive system. The proglottids are classified as immature, mature, or gravid (the latter are found at the end of the strobila and contain the fully developed uterus full of eggs). The uterine structure in the gravid proglottids is often used as the main criterion for identification. The eggs and/or scolex can also be used to identify a cestode to the species level (Algorithm 16.1).

Cestodes have complex life cycles that usually involve both the intermediate and definitive hosts. In some infections, humans serve as only the definitive hosts, with the adult worm in the intestine (*Diphyllobothrium latum*, *Taenia saginata*, *Hymenolepis diminuta*, and *Dipylidium caninum*). In other cases, humans can serve as both the definitive and intermediate hosts (*Taenia solium* and *Hymenolepis nana*).

Tapeworms have been recorded in writings from about 1500 BCE and are among the earliest known human parasites. Galen, in the second century, recognized *Taenia*, and other tapeworms have been recognized since the late 1500s (1). Apparently, eggs of *Diphyllobothrium* spp. have been found in Alaska and date from the 13th and 17th centuries (2).

The exact prevalence of human intestinal tapeworms is not really known, but estimates are that as many as 100 million people are infected with *T. saginata* and *T. solium* while approximately 75 million are probably infected with *H. nana*. Certainly, the presence of adult tapeworms in the intestinal tract is not life-threatening; however, infection with larval tapeworms can be very serious and can lead to death.

Diphyllobothrium latum

D. latum belongs to the pseudophyllidean tapeworm group, which is characterized by having a scolex with two bothria (sucking organs) rather than the typical four suckers seen in the *Taenia* tapeworms. The distribution of this worm is worldwide, with various increased outbreaks reported from time to time. Areas

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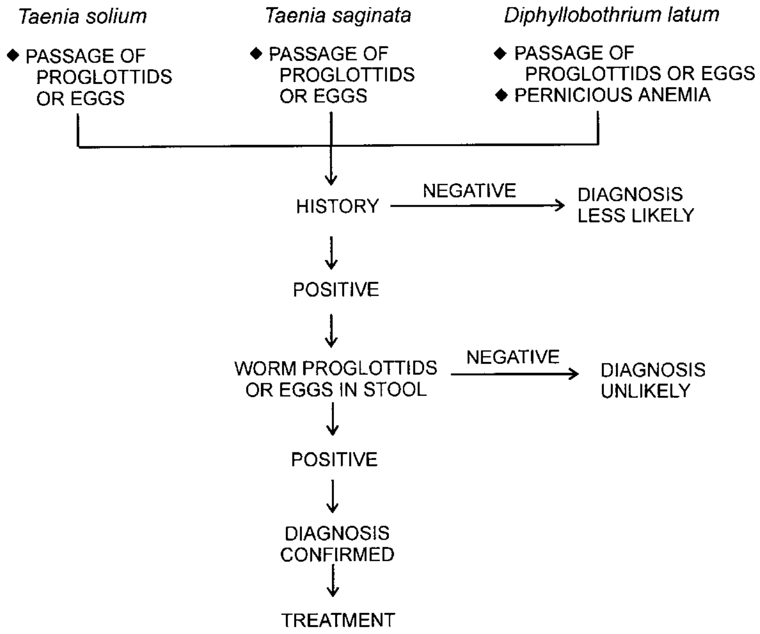


Algorithm 16.1 *Taenia solium*, *Taenia saginata*, and *Diphyllobothrium latum*.
doi:10.1128/9781555819002.ch16.Alg16.1

of the world where a high incidence of infection with *D. latum* has been reported to occur include northern Europe, Russia (Karelia, Siberia, and Far East), and North America (Canada and Alaska), but it has been recently found also in South America (Chile). Another human-infecting species, *Diphyllobothrium nihonkaiense*, seems to dominate the northern Pacific region; *D. nihonkaiense*, originally endemic in Japan, is an emerging parasite in European countries after molecular techniques were used in its diagnosis. *Diphyllobothrium pacificum* is endemic to the southern Pacific (coast of South America) and is normally found in pinnipeds off the coast of Peru.

Another widely distributed species, *Diphyllobothrium dendriticum*, has never been considered as an important or frequent parasite of humans, even though it is rather common in arctic regions. This cestode typically parasitizes arctic and subarctic piscivorous birds (such as gulls) and mammals (such as foxes or bears); human infections have been generally considered accidental. Infections have also been found in New Guinea and Australia. *Diphyllobothrium ursi* occurs in bears and humans in Alaska and Canada. Currently there are 14 species in the genus, most of which can infect humans (3).

In reviewing data from Europe during the last 20 years, results indicate that several dozen cases of diphyllobothriasis have been reported each year from Finland and Sweden, that there have been numerous cases in the French- or Italian-speaking areas around subalpine lakes, and that sporadic cases have been observed in Austria, Greece, Norway, Poland, Romania, and Spain. Over 30 cases have been identified on the Swiss shores of Lake Maggiore since 1990, and 70 cases have been reported on the Swiss and French shores of Lake Leman between 1993 and 2002. Between 8 and 12% of perch fillets from Lake Leman and 7.8% of perch from Lake Maggiore were found to contain larvae. Sources of infection include marinated fish fillets in northern Europe, "carpaccio di persico" in northern Italy, and perch and charr consumed raw or undercooked around Lake Leman (4). Certainly, the increased popularity of such foods as raw salted or marinated fillets (which originate from Baltic and Scandinavian countries), carpaccio (very thin slices of raw fish common in Italy), tartare maison (raw salmon) and poisson du lac façon nordique (in French-speaking Europe), and ceviche (lightly marinated fish in Latin America) significantly increases the risk of such infections.

The infection is acquired by the ingestion of raw, poorly cooked, or pickled freshwater or marine fish and has been associated with a condition similar to pernicious anemia (Table 16.1).

Life Cycle and Morphology

The life cycle can be seen in Fig. 16.1. Infection with the adult worm is acquired by the ingestion of raw, poorly cooked, or pickled freshwater fish (pike, perch, lawyer, salmon, trout, whitefish, grayling, ruff, turbot, etc.) and marine fish (sea fish that spawn in freshwater rivers, such as salmon) containing the encysted plerocercoid larvae. After ingestion, the worm matures, with egg production beginning in about the fifth or sixth week. The adult worm reaches a length of 15 m and may contain up to 3,000 proglottids. This is the largest known parasite in humans and can live for many years in the mammalian host.

TABLE 16.1 Cestode parasites (intestinal)

Characteristic	*Diphyllobothrium latum*	*Taenia saginata*	*Taenia asiatica*	*Taenia solium*	*Hymenolepis (Rodentolepis) nana*	*Hymenolepis diminuta*	*Dipylidium caninum*
Intermediate hosts (common)	Two: copepods and fish	One: cattle	One: pig, cattle, goats	One: pig	One: various arthropods (beetles, fleas) or none	One: various arthropods (beetles, fleas)	One: various arthropods (fleas, dog lice)
Mode of infection	Ingestion of plerocercoid (sparganum) in flesh of infected fish	Ingestion of cysticercus in poorly cooked or raw infected beef	Ingestion of cysticercus in infected tissues, primarily raw liver	Ingestion of cysticercus in poorly cooked or raw infected pork	Direct ingestion of eggs or ingestion of cysticercoid within infected arthropod; autoinfection may also occur	Ingestion of cysticercoid in infected arthropod	Ingestion of cysticercoid in fleas, lice
Prepatent period	3–5 wk	10–12 wk	10–12 wk	5–12 wk	2–3 wk	3 wk	3–4 wk
Normal life span	Up to 25 yr	Up to 25 yr	Up to 25 yr	Up to 25 yr	Perhaps many years owing to autoinfection	Usually, 1 yr	Usually, 1 yr
Length	4–10 m	4–12 m	4–8 m	1.5–8 m	2.5–4.0 cm	20–60 cm	10–70 cm
Scolex	Spatulate, 3 by 1 mm; no rostellum or hooklets; has two shallow grooves (bothria)	Quadrate, 1–2 mm in diam; no rostellum or hooklets; four suckers	Quadrate, 1–2 mm in diam; has rostellum but no hooklets; four suckers	Quadrate, 1 mm in diam; has rostellum and hooklets; four suckers	Knoblike but not usually seen; has rostellum and hooklets; four suckers	Knoblike but not usually seen; has rostellum but no hooklets; four suckers	0.2–0.5 mm in diam; has conical/retractile rostellum armed with four to seven rows of small hooklets; four suckers

Usual means of diagnosis	Ovoid, operculate yellow-brown eggs (58–75 µm by 40–50 µm) in feces; egg usually has small knob at abopercular end; proglottids may be passed, usually in chain of segments (few centimeters to 0.5 m long); proglottids wider than long (3 by 11 mm) and have rosette-shaped central uterus	Gravid proglottids in feces; they are longer than wide (19 by 17 mm) and have 15 to 20 lateral branches on each side of the central uterine stem; they usually appear singly; spheroidal yellow-brown, thick-shelled eggs (31–43 µm in diam) containing an oncosphere; may be found in feces	Gravid proglottids in feces; they are longer than wide (10 by 4 mm) and have ≥20 lateral branches on each side of central uterine stem; spheroidal yellow-brown, thick-shelled eggs (31–43) µm in diam) containing an oncosphere; may be found in feces	Gravid proglottids in feces; they are longer than wide (11 by 5 mm) and have 7 to 13 lateral branches on each side of central uterine stem; usually appear in chain of five to six segments; spheroidal yellow-brown, thick-shelled eggs (31–43 µm) containing an oncosphere; may be found in feces	Nearly spheroidal pale, thin-shelled eggs (30–47 µm in diam) in feces; oncosphere surrounded by rigid membrane, which has two polar thickenings from which four to eight filaments (polar filaments) extend into the space between the oncosphere and the thin, outer shell	Large, ovoid, yellowish, moderately thick-shelled eggs (70–85 µm by 60–80 µm) in feces; egg contains an oncosphere but no polar filaments	Gravid proglottids (8–23 mm long) containing compartmented clusters of eggs in feces; proglottids have genital pores at both lateral margins; occasionally individual oncospheres (20–33 µm in diam) visible in feces
Diagnostic problems or notes	Eggs are sometimes confused with eggs of *Paragonimus* spp.; eggs are unembryonated when passed in feces	Eggs are identical to those of *T. solium*; ordinarily can distinguish between species only by examination of gravid proglottids; eggs can be confused with pollen grains (handle all proglottids with extreme care)	Eggs are identical to those of *T. solium* and *T. saginata*; ordinarily can distinguish between species only by examination of gravid proglottids; proglottids much smaller than those of *T. saginata* or *T. solium;* eggs can be confused with pollen grains (handle all proglottids with extreme care)	Eggs are identical to those of *T. saginata*; eggs less likely to be found in feces than with *T. saginata* (handle all proglottids with extreme care as *T. solium* eggs are infective to humans)	Sometimes confused with eggs of *H. diminuta*; rodents serve as reservoir hosts	Should not be confused with *H. nana*, as eggs lack polar filaments; rodents serve as reservoir hosts; infections rare, usually in infants or children	Gravid proglottids resemble rice grains (dry) or cucumber seeds (moist); dogs and cats serve as reservoir hosts

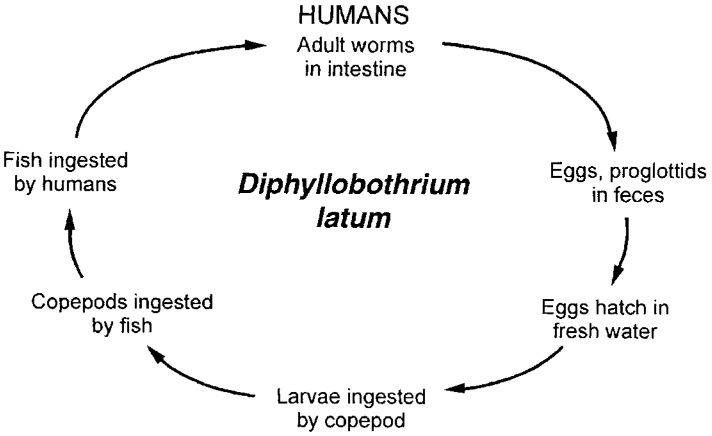
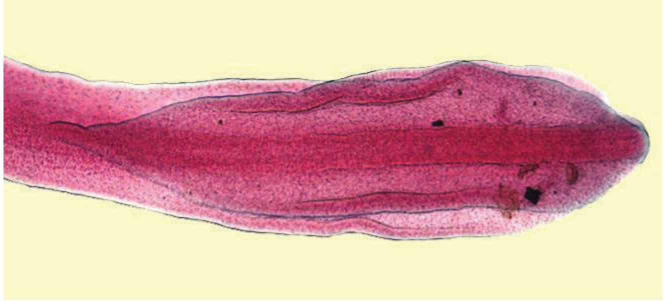

HUMANS
Adult worms in intestine
Eggs, proglottids in feces
Eggs hatch in fresh water
Larvae ingested by copepod
Copepods ingested by fish
Fish ingested by humans
Diphyllobothrium latum

Figure 16.1 Life cycle of *Diphyllobothrium latum*. doi:10.1128/9781555819002.ch16.f1

The scolex of *D. latum* is elongate and spoon shaped and has two long sucking grooves, one on the dorsal surface and the other on the ventral surface (Fig. 16.2). The mature and gravid proglottids are wider than they are long, with the main reproductive structures (mainly the uterus) located in the center of the gravid proglottid. This configuration of the uterine structure has been called a rosette (Fig. 16.3 and 16.4). Identification to the species level is usually based on this typical morphology of the gravid proglottids. Both eggs and proglottids may be found in the stool. Often, a partial chain of proglottids may be passed (a few inches to several feet).

The eggs are broadly oval and operculated and measure 58 to 75 μm by 40 to 50 μm (Fig. 16.5). After developing for 2 weeks in freshwater, the eggs hatch and the ciliated, coracidium larvae are ingested by the first intermediate host, the copepod (Fig. 16.6). The copepods, containing the second larval stage (procercoid), are then ingested by fish, which may be ingested by larger fish. In this situation, the final fish intermediate host may contain many plerocercoid larvae, which initiate the infection with the adult worm when ingested by humans (Fig. 16.7).

Figure 16.2 Scolex of *Diphyllobothrium latum*. doi:10.1128/9781555819002.ch16.f2

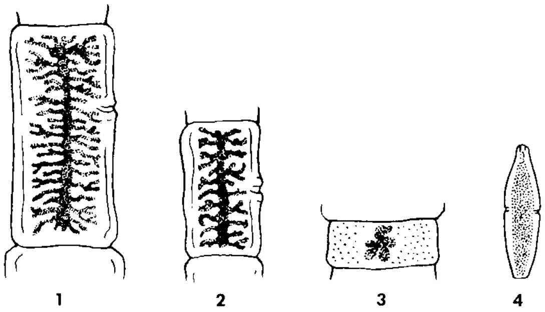


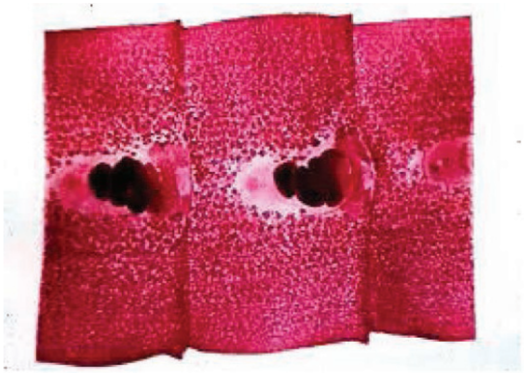
Clinical Disease

Symptoms depend on a number of variables: the number of worms present, the amounts and types of by-products produced by the worm, the patient's reaction to such by-products, and the absorption of various metabolites by the worms. Occasionally intestinal obstruction, diarrhea, abdominal pain, or anemia occurs. If the worm is attached at the jejunal level, there may be a vitamin B_{12} deficiency, which resembles pernicious anemia and develops in a very small percentage of persons harboring the tapeworm. Heavy or long-term infections with *D. latum* may cause megaloblastic anemia due to parasite-mediated dissociation of the vitamin B_{12}-intrinsic factor complex within the gut lumen, making B_{12} unavailable to the host (5). This clinical picture is seen most frequently in Finland, where individuals tend to have a genetic predisposition to pernicious anemia. In patients without this genetic predisposition, symptoms of a *D. latum* infection may be absent or minimal, consisting of a slight leukocytosis with eosinophilia.

Most patients are actually asymptomatic; however, the following symptoms were found more frequently in those who were infected than in the control group: fatigue, diarrhea, dizziness, weakness, numbness of extremities, and a

Figure 16.3 Gravid proglottids. (1) *Taenia saginata*; (2) *T. solium*; (3) *Diphyllobothrium latum*; (4) *Dipylidium caninum*. doi:10.1128/9781555819002.ch16.f3





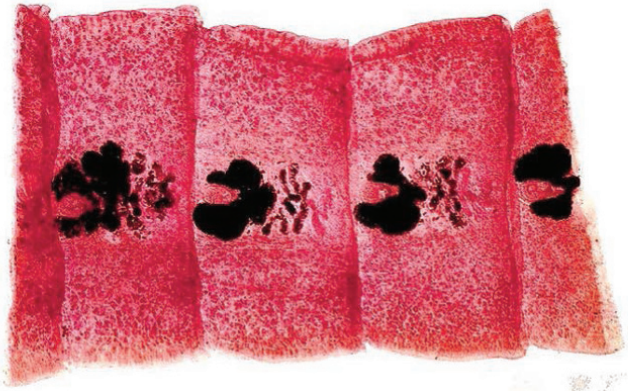


Figure 16.4 Gravid proglottids of *Diphyllobothrium latum* (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch16.f4

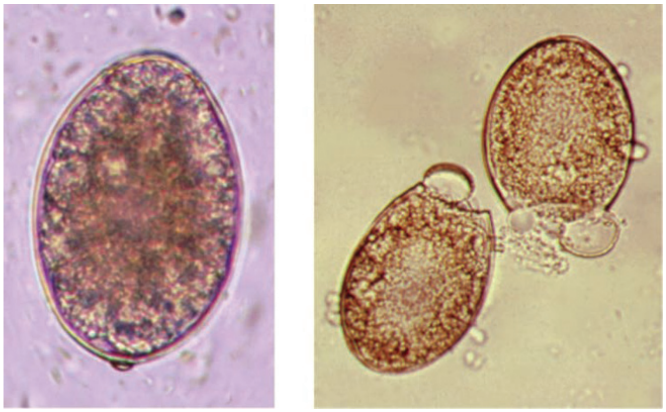


Figure 16.5 *Diphyllobothrium latum* eggs. (Left) Note the small knob at the abopercular end (end opposite the operculum); also note that the operculum does not fit into "shoulders" like the *Paragonimus* eggs but presents a smooth outline on the egg shell. (Right) Note the open opercula; this can be accomplished by tapping on the coverslip (wet mount) to get opercula to pop open. doi:10.1128/9781555819002.ch16.f5

sensation of hunger. No differences were found in terms of abdominal pain. Symptoms occur in approximately 22% of infections. No single proglottids are routinely passed in the stool as is seen with *Taenia* infections, although occasionally a string of proglottids is passed or vomited.

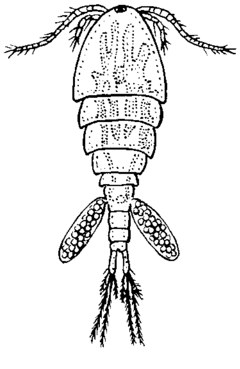
About 40% of patients infected with *D. latum* have reduced vitamin B_{12} levels, but fewer than 2% develop anemia. In cases of severe anemia, there may be neurologic symptoms such as weakness, numbness, paresthesia, movement and coordination problems, and impairment of deep sensibilities. Both the anemia and neurologic problems are reversed by administration of vitamin B_{12} and do not reoccur after the worm has been eliminated. This type of severe anemia is rarely seen, probably as a result of overall improvements in general nutrition of the population in the area of endemic infection.

Diagnosis

Diagnosis is usually based on the recovery and identification of the characteristic eggs or proglottids. If the egg operculum is difficult to see, the coverslip of the wet preparation can be tapped and the pressure may cause the operculum to pop open, thus making it more visible. The eggs are unembryonated at the time they are passed in the stool. Proglottids are often passed in chains (a few inches to several feet), and this is a clue to *D. latum*. The overall proglottid morphology with the rosette uterine structure also facilitates identification.

Although differentiation is not generally a problem, the eggs of *D. latum* look very much like those of *Nanophyetus salmincola*, a trematode that is commonly found in the Pacific Northwest region of the United States and in eastern Siberia. *N. salmincola* eggs have been described as being broadly oval, operculate, relatively thick-shelled, and

Figure 16.6 Copepod. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch16.f6



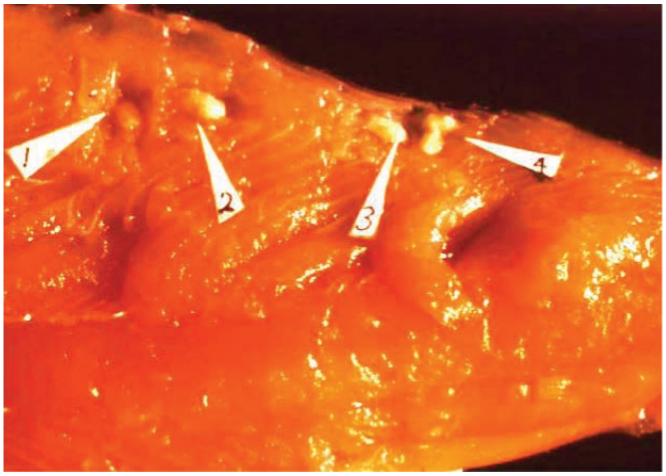


Figure 16.7 *Diphyllobothrium latum*. Encysted pleurocercoids in cherry salmon fillet. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of T. Oshima, Yokohama City University.) doi:10.1128/9781555819002.ch16.f7

yellowish; they measure 60 to 80 μm by 34 to 50 μm, a direct overlap with the size range of *D. latum* (58 to 75 μm by 40 to 50 μm). However, human infection with *N. salmincola* is generally incidental and uncommon.

In patients with vague abdominal complaints or unexplained low vitamin B_{12} levels, it might be worth inquiring about the types of fish consumed, particularly including sushi and sashimi, and it might be worth checking the stool for *Diphyllobothrium* ova.

Identification to the species level of broad tapeworms (genus *Diphyllobothrium*) infecting humans is very difficult to achieve using morphology only. Molecular analysis by PCR and sequencing represents the only reliable tool to date to identify these parasites to the species level. From a medical perspective, *D. latum*, *D. nihonkaiense*, *D. dendriticum*, and *D. pacificum* are the most important, because humans represent their preferred definitive hosts (6). However, molecular methods are rarely used in routine laboratories due to economical and technical reasons. Also, all human-infecting *Diphyllobothrium* species cause similar symptoms, and infections are successfully treated with praziquantel. Thus, some think that a specific identification of the causative agent is not necessary. However, the specific diagnostic identification of *Diphyllobothrium* isolates is important from an epidemiological point of view. Human diphyllobothriasis has been estimated to affect 20 million people worldwide (6), and recent studies indicate a recrudescence in some well-developed countries. Thanks to the use of molecular tools, locally acquired infections with allochtonous species are being reported more and more (7).

KEY POINTS—LABORATORY DIAGNOSIS

Diphyllobothrium latum

1. Careful examination of the eggs should reveal the operculum. If it is difficult to see, it can sometimes be popped open by tapping on the coverslip of the wet preparation. The light should be somewhat reduced to allow the operculum to be seen more easily.
2. Often, the proglottids are passed in chains (a few inches to a few feet). Also, the gravid proglottids are much wider than long, with the uterine structure being seen in the middle of the proglottid (rosette).
3. Handling of the specimens presents no danger in terms of cysticercosis; however, all fecal specimens should be considered potentially infectious (with other organisms).

Treatment

The use of both praziquantel and niclosamide has been recommended (see chapter 36). A single dose of praziquantel (5 to 10 mg/kg of body weight) for both adults and children eliminates the infection in >90% of patients. Another option is 10 to 25 mg/kg of praziquantel given as a single dose (6). Niclosamide is given as a single dose of 2 g (four tablets chewed thoroughly). A single dose of 50 mg/kg is recommended for children. Niclosamide may not be available within the United States. After therapy, if the worm has not been spontaneously passed within 2 h, a saline purgation can be used.

Epidemiology and Prevention

Although *D. latum* infections have been detected in other mammals, in areas where human infection has become rare, the natural transmission cycle from mammals other than humans does not seem to be sustained. It is quite possible to acquire the infection from the ingestion of infected raw freshwater fish that has been shipped under refrigeration to areas where the infection is not endemic. Preventive measures would include thorough cooking of all freshwater fish or freezing for 24 to 48 h at −18°C. This infection has been called the Jewish housewives' disease, since the individual preparing the food may sample the dish (e.g., gefilte fish) prior to cooking and acquire the infection. Other groups who tend to eat raw or insufficiently cooked fish include the Russians, Finns, and Scandinavians (8). Raw fish marinated in lime juice (ceviche) is also a source of infection (*D. pacificum*) in Latin America. Since the domestic dog can serve as a reservoir host, infected dogs should be periodically treated. Other factors contributing to the parasitic cycle include the continued dumping of wastewater into lakes and possible animal reservoirs.

Cooking fish at a temperature of only 55°C for 5 minutes will kill the larvae; freezing to −20°C for 7 days, or flash freezing to –35°C for 15 hours, as long as the flesh is less than 15 cm thick, effectively kills the larvae also (6). Sushi and sashimi chefs should use only fish frozen in this way. While these freezing protocols are the standards required by public health regulations, they are not easy for public health inspectors to verify.

Taenia solium

T. solium infection may have been recognized since biblical times, and the life cycle was delineated in the mid-1850s (8). The tapeworm is found in many parts of the world and is considered an important human parasite where raw or poorly cooked pork is eaten (Table 16.1).

Cysticercosis infections with *T. solium* larvae are relatively common in certain parts of the world. However, cysticercosis is becoming more common within the United States; the infection is associated with Hispanic ethnicity, immigrant status, exposure to areas where neurocysticercosis is endemic, and the American Southwest (9, 10). Serum immunoblotting shows that the seroprevalence of cysticercosis and infection with the adult tapeworm in a rural southern California population approximates the prevalence in some areas of Latin America where the infection is endemic (9). Also, an increasing number of cases have been reported in the United States over the past 50 years (10). This extraintestinal form of the infection is far more serious than the presence of the adult worm in the intestine.

Life Cycle and Morphology

The life cycle can be seen in Fig. 16.8. Infection with the adult worm is initiated by the ingestion of raw or poorly cooked pork containing encysted *T. solium* larvae (Fig. 16.9). The larva is digested out of the meat in the stomach, and the tapeworm head evaginates in the upper small intestine, attaches to the intestinal mucosa, and grows to the adult worm within 5 to 12 weeks. Although usually a single worm is present in the intestine, multiple worms may occur. The adult worm reaches a length of 2 to 7 m and may survive for 25 years or more (8).

The attachment organ, or scolex, has four large suckers with a rounded rostellum containing a double row of 25 to 30 small hooks (Fig. 16.10). The neck region is short and about half the width of the scolex. The total number of proglottids is less than 1,000, with the immature proglottids being wider than long, the mature proglottids being approximately square, and the gravid segments being longer than wide.

Identification to the species level is usually based on the number of lateral uterine branches in the gravid proglottid (Fig. 16.3 and 16.11). The main lateral branches should be counted where they come off the main central stem. Only one side is counted, and there are between 7 and 13 branches, with an average of 9.

The terminal gravid proglottids break off from the main strobila and are passed out in the stool or actually migrate out of the bowel. In some cases, three or four attached proglottids pass out in the stool; this is highly suggestive of *T. solium* rather than *T. saginata*, which usually passes out as single proglottids. Eggs usually pass from the uterus through the ruptured wall, where the proglottids break off from the strobila. The eggs are round or slightly oval (31 to 43 µm), have a thick, striated shell, and contain a six-hooked embryo or oncosphere (Fig. 16.12 and 16.13). These eggs may remain viable in the soil for weeks.

On ingestion by hogs or humans, the eggs hatch in the duodenum or jejunum after exposure to gastric juice in the

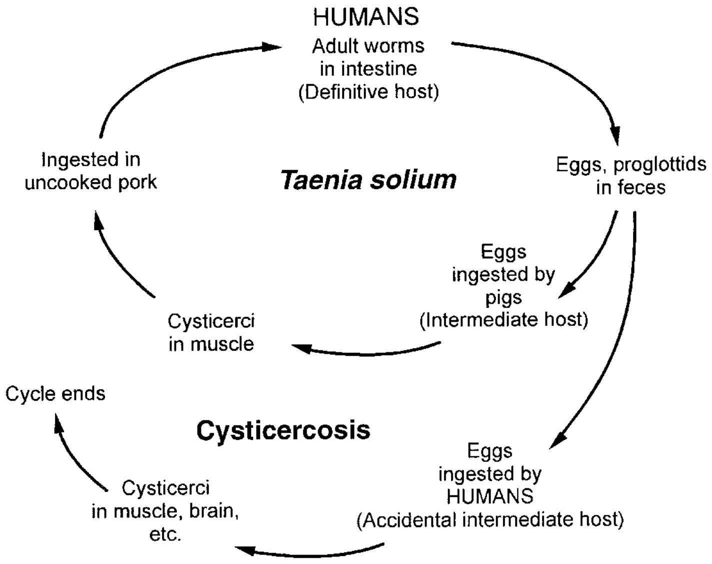


Figure 16.8 Life cycle of *Taenia solium*.
doi:10.1128/9781555819002.ch16.f8

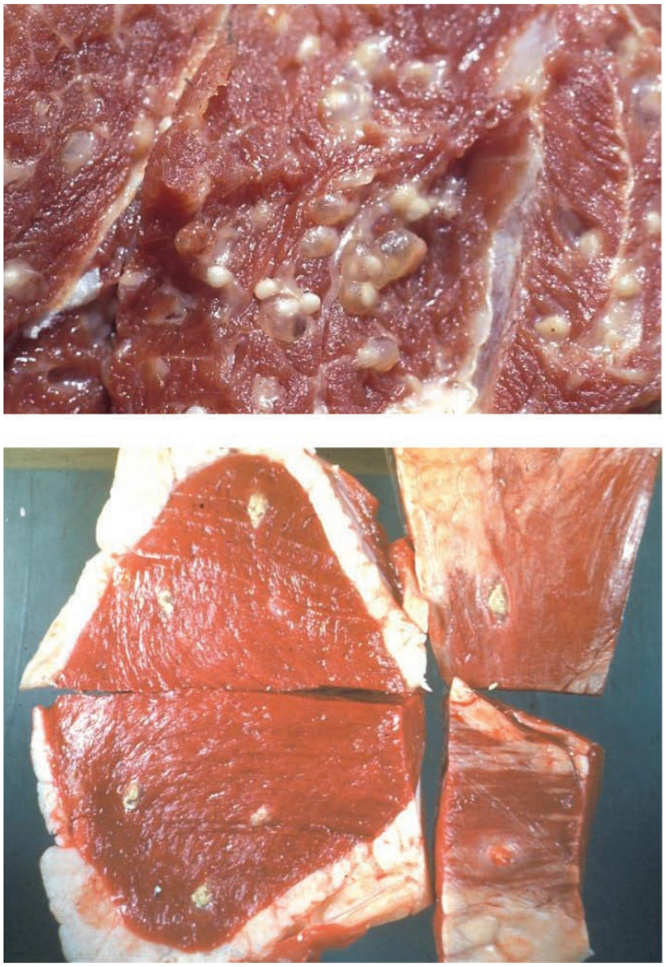


Figure 16.9 Cysticerci in muscle. (Upper) Pork; (lower) beef. (Courtesy of the Iowa State University Department of Veterinary Pathology, Center for Food Security and Public Health, Armed Forces Institute of Pathology, and Plum Island Animal Disease Center.) doi:10.1128/9781555819002.ch16.f9

stomach. The released oncospheres penetrate the intestinal wall, are carried via the mesenteric venules throughout the body, and are filtered out in the subcutaneous and intramuscular tissues, the eyes, the brain, and other body sites. This cysticercus is an ovoid, milky-white bladder with the head invaginated into the bladder (Fig. 16.14). The bladder worms measure 5 mm long by 8 to 10 mm wide; however, in the brain, they may reach a volume of 60 ml. The host generally produces a fibrous capsule around the bladders, unless they are located in the brain, particularly the ventricles.

Clinical Disease

Adult Worm. The presence of the adult worm usually causes no problems other than slight irritation at the site of attachment or vague abdominal symptoms (hunger pains, indigestion, diarrhea, and/or constipation). There may be a low-grade eosinophilia, usually below 15%, and increased levels of IgE in serum.

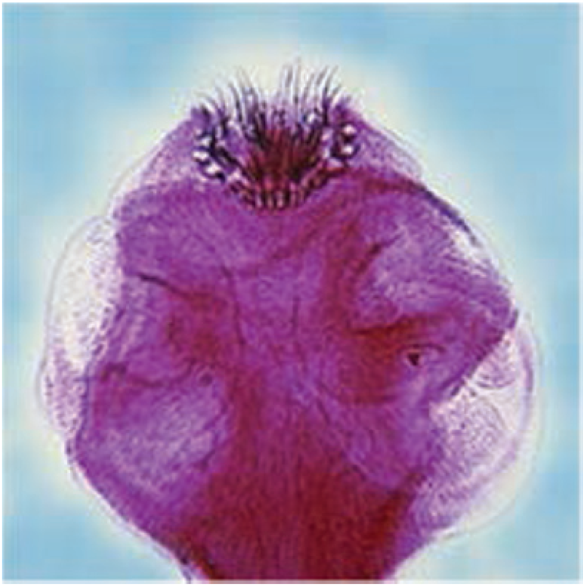


Figure 16.10 Scolex of *Taenia solium*. doi:10.1128/9781555819002.ch16.f10

Cysticercosis. The larval forms of *T. solium* have been called *Cysticercus cellulosae*; however, this term is not taxonomically correct and is used infrequently. These cysticerci have been recovered from all areas of the body, and symptoms depend on the particular body site involved. The presence of cysticerci in the brain represents the most frequent parasitic infection of the human nervous system and the most common cause of adult-onset epilepsy throughout the world (11). For unknown reasons, in Latin America it is unusual for both brain and muscle cysticercosis to occur in the same patient, with fewer than 6% of patients having cysticerci in both sites. However, elsewhere in the world, subcutaneous involvement by *T. solium* cysticerci has been detected in as many as 78.5% of patients with cerebral cysticercosis (12). Possible reasons for such differences may include (i) the immune status of the patient, (ii) the human leukocyte antigen type, (iii) the nutritional status of the patient, (iv) the burden of eggs infecting the patient, and (v) a difference in the strains of *T. solium*. It is also rare for a patient with cysticercosis to harbor adult *T. solium* worms in the intestine. Neurocysticercosis usually affects males and females of all ages, with a peak incidence between 30 and 50 years of age. It has been estimated that 50,000 individuals die annually due to neurocysticercosis.

Neurocysticercosis is the most common parasitic disease of the central nervous system; in some countries the prevalence exceeds 10% and it is responsible for up to

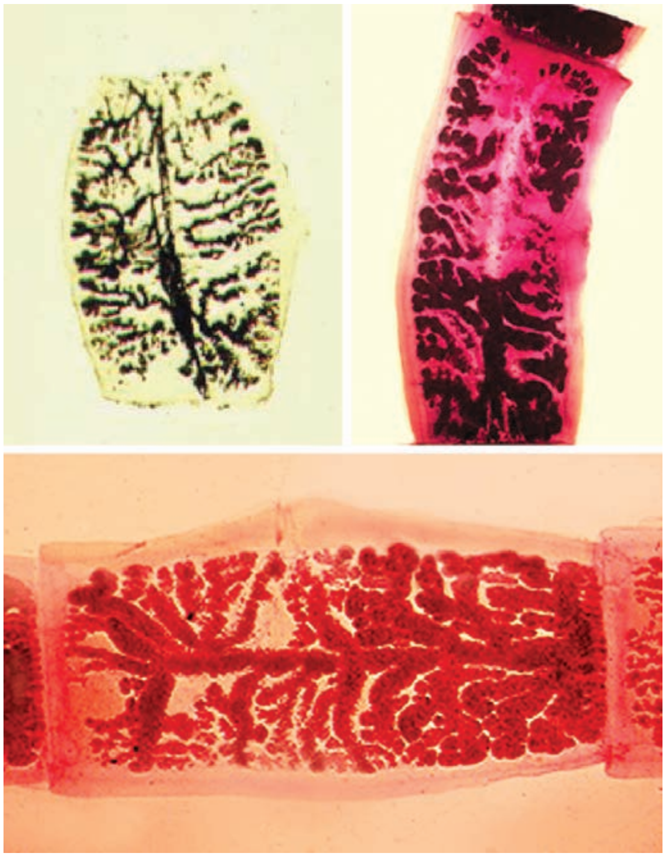


Figure 16.11 Gravid proglottids of *Taenia solium*. (Upper, left) India ink injection of gravid proglottid; (right) India ink injection of gravid proglottid with carmine overlay. (Lower) Oil Red O stain of gravid proglottid. (Upper right and lower courtesy of the CDC Public Health Image Library, Mae Melvin.)
doi:10.1128/9781555819002.ch16.f11

50% of late-onset epilepsy cases (13). Factors related to neurocysticercosis pathology include (i) the individual immune response to the presence of the parasite, from tolerance to a severe inflammatory response; (ii) the location of the parasite in the brain; (iii) the number of cysticerci present; and (iv) the stage or age of the lesions (live cysts, inflammatory exudates, granulomas, calcifications, and residual fibrosis). Estimates of prevalence as defined in Latin American countries range between 15% and 38%. Applying these prevalence calculations to the 75 million persons at risk in these geographic areas in 1993, it is estimated that between 11 million and 29 million persons there have cysticercosis (14).

When the larvae are found in the brain, symptoms can result from actual larval invasion of the brain tissue and/or death of the organism, which stimulates tissue reactions around the larvae (Fig. 16.15 and 16.16). It has been recommended that in every case of epilepsy occurring in a patient with no family history of fits and no personal

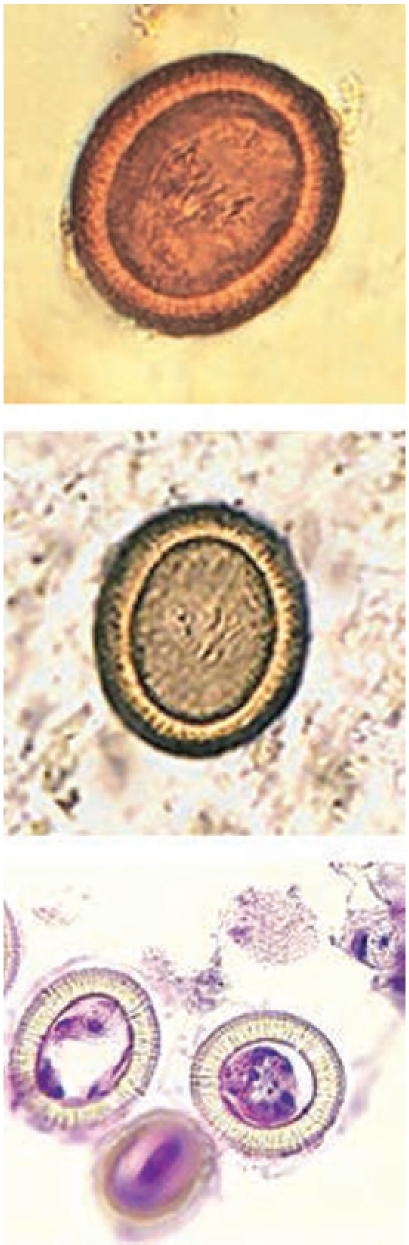


Figure 16.12 *Taenia* eggs. (Top and middle) Note the striated shell and the presence of the six-hooked oncosphere within the thick egg shell. The hooks may not be visible in every egg, particularly in specimens that have been stored for long periods in preservatives; however, in clinical specimens submitted to the laboratory in fixatives for timely examination, the hooklets are generally visible within the egg shell. Without special staining, the eggs of *Taenia* spp. cannot be identified to the species level (*T. solium*, *T. saginata*, *T. asiatica*). (Bottom) Eggs of *Taenia* sp. in a gravid proglottid, stained with hematoxylin and eosin (H&E). Note the characteristic striations, typical for the taeniids. Not visible in these images are the hooks commonly seen in cestode eggs. Hooks do not stain with H&E but are refractile. (Courtesy of the CDC Public Health Image Library.)
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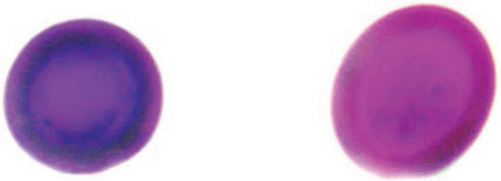

Figure 16.13 *Taenia* eggs. (Left) *Taenia solium*; (right) *Taenia saginata*. Ziehl Neelsen staining can occasionally distinguish fully mature *T. solium* from *T. saginata* eggs, in cases where the cover is entirely magenta (*T. saginata*) or entirely blue/purple (*T. solium*). Staining can be mixed or equivocal. While this distinction provides a species diagnosis in 35% of cases, it is certainly not absolute and not very practical. Also, use of this technique assumes that eggs are fully mature (which is not possible to determine in a proglottid) and is relatively subjective since color differences may be subtle. (Courtesy of Jimenez JA et al, *Trop Med Int Health* **15:**1077–1081, 2010.) doi:10.1128/9781555819002.ch16.f13

Figure 16.14 (A) Cysticercus with head invaginated into the bladder (courtesy of Christina Thoisen, Department of Veterinary Disease Biology, University of Copenhagen, Denmark). (B) Scanning electron micrograph of scolex from cysticercus (photograph courtesy of U.S. Department of Agriculture).
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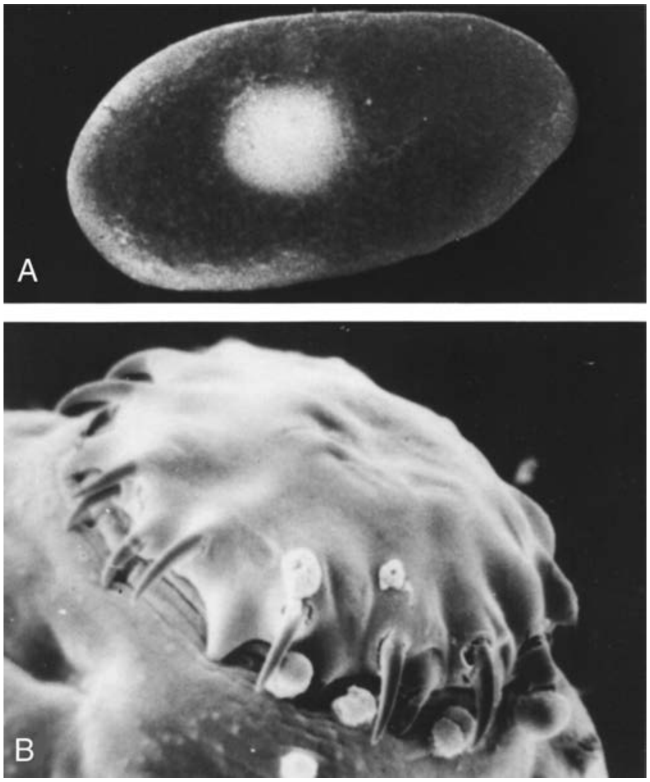


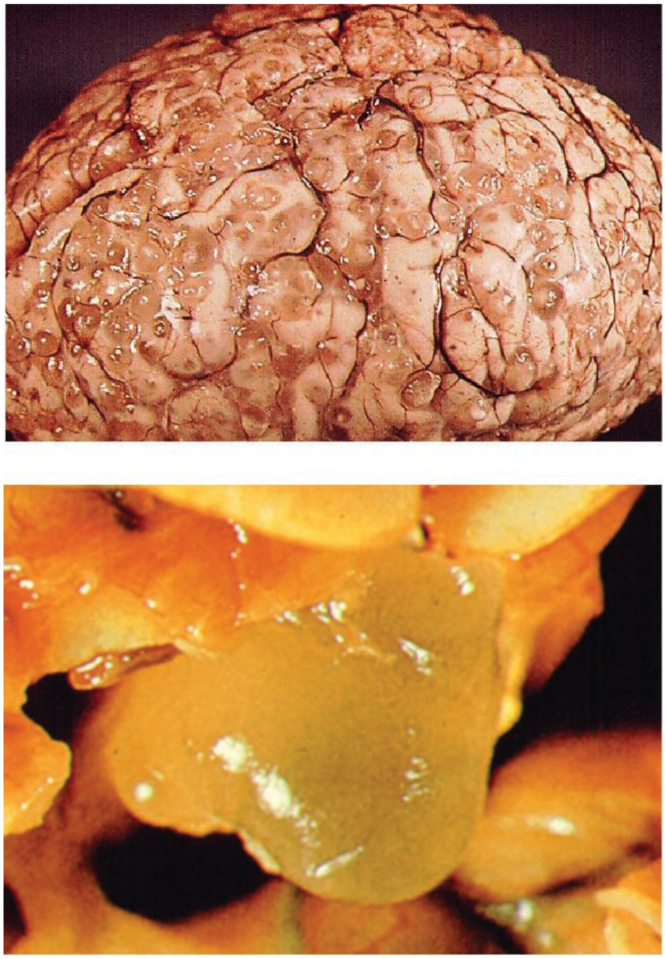


Figure 16.15 (Upper) Gross specimen of brain containing many cysticerci. (Lower) Single cysticercus in the brain. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch16.f15

history of fits in childhood, the probability of cysticercosis should be entertained (15). Although studies indicate the presence of epileptiform seizures in patients with cysticercosis, other symptoms may also be present, including abnormal behavior, transient paresis, intermittent obstructive hydrocephalus, disequilibrium, meningoencephalitis, and visual problems. Spinal fluid from patients with cerebral cysticercosis shows possible eosinophilia and pleocytosis (the most common cell type is the lymphocyte), with atypical mononuclear lymphoid cells. The most distinctive features of the infection in the spinal fluid are atypical, reactive lymphoid cells with a mixed cellular population. A study by Sotelo et al. of 753 cases of neurocysticercosis reviewed inactive and active disease, the frequency of signs and symptoms, and cerebrospinal fluid (CSF) findings. More than 50% of all patients exhibit at least two of these clinical presentations (16) (Table 16.2). Although rare, the

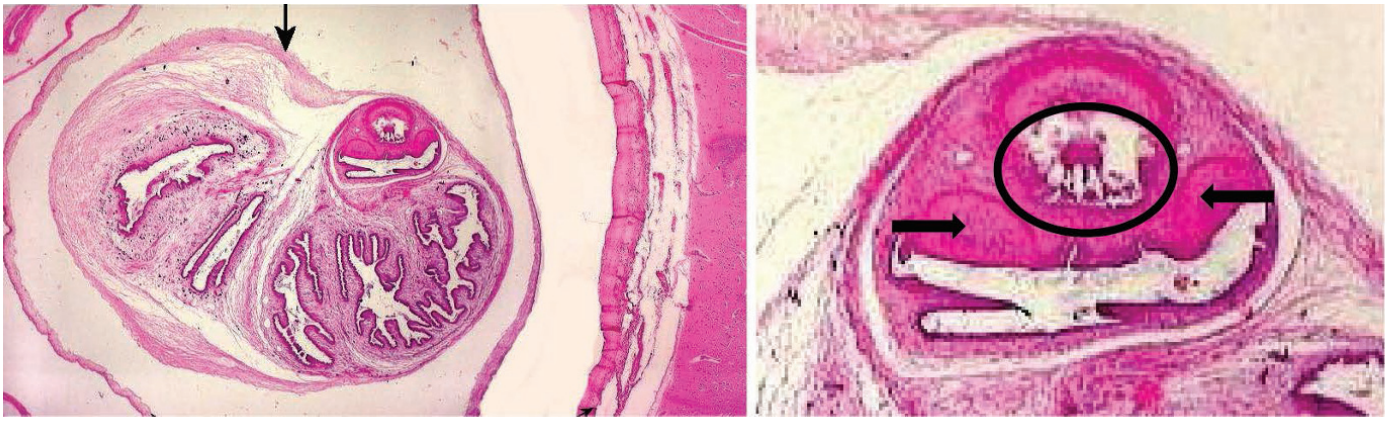


Figure 16.16 (Left) Single cysticercus from brain (arrow). (Right) Note the suckers (arrows) and hooklets (circle). (Courtesy of Luciano S. Queiroz, Dept. of Pathology, School of Medical Sciences, State University of Campinas [UNICAMP], Campinas, Sao Paulo, Brazil; gradanat@fcm.unicamp.br.) doi:10.1128/9781555819002.ch16.f16

occurrence of secondary parkinsonian tremors in previously shunted patients with cysticercosis suggests obstruction and requires immediate evaluation (17).

In a study from Mexico, the frequency of cerebral arteritis in subarachnoid cysticercosis was higher than previously reported. Involvement of middle-sized vessels is a common occurrence, even in patients without clinical evidence of ischemia (18).

Although parenchymal cysts are the most common location in the brain, cysts may also be present in the ventricles, meninges, spinal cord, and subarachnoid spaces. Cysticerci have been recovered from the brain (all areas), spinal cord, breast, thyroid, eye (orbit, subretinal), muscles (various sites), mouth, salivary gland, tongue, lip, masseter muscle, pharynx, nose, kidney, lymph nodes, pituitary gland, hypothalamus, parotid gland, submandibular, thorax, scrotum, heart, and subcutaneous tissue and from disseminated infections.

Although rare, a case of acute leukemia and massive cardiopulmonary cysticercosis has been reported (19). In this case, pulmonary cysticercosis may have been caused by the immunosuppression.

Cysticerci in the eye can be found in various areas, including in the eyelid and under the conjunctiva. The larvae are unencapsulated when found in the vitreous or anterior chamber and will actively change shape, casting shadows in front of the retina (Fig. 16.17). Unless the organism is removed, permanent eye damage may occur, particularly if the parasite dies, thus stimulating an intense inflammatory reaction.

A single case of cysticercosis involving the scrotum has been reported; the patient's presentation was that of testicular torsion requiring surgical exploration. The pa-

TABLE 16.2 Classification of neurocysticercosis[a]

Form	Occurrence in 753 cases (%)
Active	
Arachnoiditis[b] (headache, vertigo, vomiting, cranial nerve dysfunction)	46.2
Hydrocephalus[c] secondary to meningeal inflammation (intracranial hypertension, ataxic gait, intellectual deterioration)	25.7
Cysts within brain itself (epilepsy, headache)	13.2
Brain infarction secondary to vasculitis (unilateral pyramidal signs, intracranial hypertension, psychiatric disturbances)	2.3
Mass effect due to a large cyst or clump of cysts (tumorlike signs, epilepsy)	1.0
Inactive	
Granulomas or calcifications within the brain (epilepsy, usually partial seizures)	57.6
Hydrocephalus[c] secondary to meningeal fibrosis (intellectual deterioration, cranial nerve dysfunction, intracranial hypertension, Parinaud's sign[d])	3.8

[a] Adapted from reference 16.
[b] Inflammation of the intermediate membrane (between pia mater and dura mater) which encloses the brain and spinal cord.
[c] Increased accumulation of cerebrospinal fluid within the ventricles of the brain, usually caused by interference with normal circulation of fluid.
[d] Paralysis of conjugate upward movement of the eyes without paralysis of convergence; associated with lesions of the midbrain.

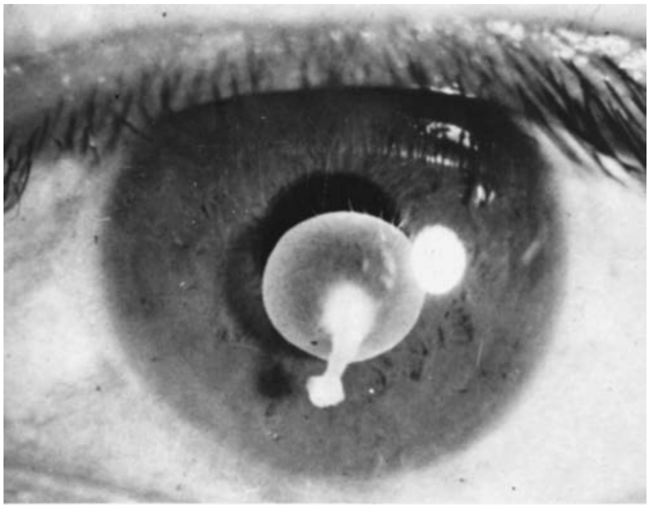


Figure 16.17 Cysticercus in the eye. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of R. Delgado y Garnica, Hospital Infantil de Tacubaya, Arq. Carlos Laxo y Gaviota, Mexico D.F.) doi:10.1128/9781555819002.ch16.f17

tient was a 9-year-old Hispanic boy who was born and raised in the United States and had no history of foreign travel. Histologic testing was confirmatory, as were the serologic results (20).

Based on fine-needle aspiration cytology, five cases of cysticercosis of the tongue and buccal mucosa in children have been reported. Symptoms included gradually increasing nodular swelling; cysticercosis was not considered in any of these cases. Since patients with these lesions may be seen by a dentist rather than a physician, cysticercosis should be included in the differential diagnosis of solitary nodular lesions in the oral cavity, particularly in young patients, in areas of endemic infection (21). In another series of patients, 21 lesions were seen in 16 Latin American patients. Most cases appeared as asymptomatic submucosal nodules that resembled mucous cysts or benign mesenchymal neoplasms. They were found in the tongue (11 cases), followed by buccal mucosa and the lower lip (4 cases each) and upper lip (2 cases) (22).

There appear to be cases where reactivation of neurocysticercosis occurs, often several years after the patient was considered cured. Several years previously, one patient had undergone treatment and was presumed to be cured of active disease. Computed tomographic (CT) and magnetic resonance imaging (MRI) studies performed 3 months prior to presentation revealed multiple intracerebral calcified lesions consistent with resolved neurocysticercosis. The patient presented with frontal headaches that occurred intermittently and were associated with vomiting, slowed mentation, and episodic visual scintillations. On presentation, additional imaging studies were performed and showed the same calcified lesions; however, these lesions were now surrounded by large areas of edema (23). Although this patient's symptoms resolved spontaneously within several days, she received a 28-day course of albendazole, cimetidine, and dexamethasone, with subsequent resolution of the cerebral edema. Another earlier report describes five patients whose CT scans showed an area of brain swelling surrounding previously inactive calcifications (24). There are several possible explanations for these relapse cases. Cerebral edema may have resulted from the death of worms that were incompletely calcified and still viable. There may have been antigen release from dead, calcified parasites. Another explanation, which might be more realistic, suggests that a spontaneous increase in the immune response could have been triggered by antigen release from live or dying parasites. Based on these cases, it is important to remember that patients with calcified lesions may present with late-onset symptoms associated with marked inflammation and cerebral edema surrounding these lesions (25). Retreatment with anthelmintics may or may not be required; corticosteroid therapy may be sufficient.

Larger numbers of cysticerci usually correlate with more serious disease. In general, patients with >20 cysticerci within the parenchyma and the absence of hydrocephalus have a more positive prognosis than do patients who have many basal or ventricular lesions accompanied by hydrocephalus. Also, it is not clear how many asymptomatic patients with intracranial cysticerci eventually develop symptoms or how many symptomatic patients spontaneously recover.

Cysticercus, Racemose Type. The racemose, or proliferating, form of the cysticercus is composed of several bladders that are connected and are often found in the brain, particularly the fourth ventricle and subarachnoid space (Fig. 16.18). Within these bladders, no scolices are found and the growth may resemble that of a metastatic tumor. Other larval cestodes may also have a racemose growth pattern, and it may be very difficult to differentiate a *T. solium* racemose cysticercus from other types histologically. The prognosis for such infections is very poor. Racemose cysticerci are usually not found in infected children and may be either a degenerative form or formed in response to infection at a different anatomic site. Cervical spinal cysticercosis is usually associated with the racemose type within the posterior fossa.

Diagnosis of racemose cysticercosis is based on the combination of clinical, epidemiologic, radiographic, and immunologic information. Compared with the more normal presentation of cysticercosis that most commonly presents as seizures, due to its extraaxial location, racemose

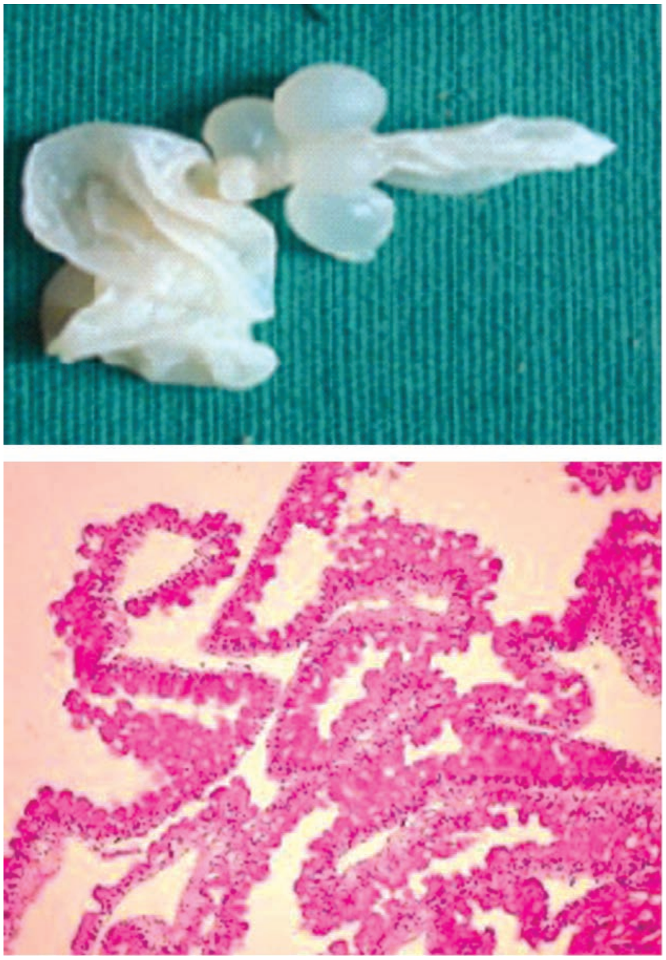


Figure 16.18 (Upper) Racemose form of *T. solium* cysticercus with several interconnected bladders of various sizes (gross specimen). [Courtesy of Velho V et al, *Internet J Neurosurg* 7(1), 2009, https://ispub.com/IJNS/7/1/9907.] (Lower) Section of proliferating bladder wall of racemose cysticercus, demonstrating multiple layers. (Courtesy of Luciano S. Queiroz, Dept of Pathology, School of Medical Sciences, State University of Campinas [UNICAMP], Campinas, Sao Paulo, Brazil; gradanat@fcm.unicamp.br.) doi:10.1128/9781555819002.ch16.f18

neurocysticercosis presents with raised intracranial pressure and meningitis and frequently requires neurosurgical intervention. Racemose cysticercosis is rare in the cerebellar hemisphere but neurocysticercosis should be taken into consideration as a differential diagnosis of multiple cystic lesions in the cerebellum.

Although infection with both HIV and *T. solium* would be expected to occur more frequently due to the increased frequency of HIV in areas where cysticercosis is endemic, very little is known about the impact of HIV on the racemose type of cysticercosis. Very large cysts and the racemose type of neurocysticercosis appear to be more frequent in HIV-positive patients; these findings may be related to an impaired cell-mediated immune response (26). The introduction of highly active antiretroviral therapy (HAART) has decreased the mortality and morbidity associated with HIV infection. However, in some patients, immune recovery leads to an inflammatory condition termed immune reconstitution inflammatory syndrome; this presents with clinical worsening of an opportunistic infection under treatment or uncovering of a subclinical infection (27). Neurocysticercosis coinfection is likely to be increasingly recognized in patients with HIV and should be included in the differential diagnosis of central nervous system infections in these patients. Consideration of the patient's immune status should alert the clinician to potential atypical presentations. Neurocysticercosis also needs to be considered in populations in areas of endemicity even when there are atypical manifestations (e.g., giant cysticerci) or lesions suggestive of other infections (e.g., enhancing lesions compatible with toxoplasmosis) (28).

Diagnosis

Adult Worms. Identification of adult worms to the species level is not possible from the eggs because *T. solium* and *T. saginata* eggs look identical. Identification is usually based on the recovery and examination of the gravid proglottids, in which the main uterine lateral branches are counted after India ink injection staining or some other clearing mechanism (7 to 13 branches for *T. solium* and 15 to 20 for *T. saginata*) (Fig. 16.3). If the scolex is recovered after therapy (purgation may be necessary to recover the intact attachment organ), the presence of the four suckers and the armed rostellum with hooks will differentiate *T. solium* from *T. saginata*. There are some other differences in the mature proglottids; however, these require staining and clearing of the proglottid and may be much more difficult to see.

It is well known that the proglottids of *T. saginata* can actively migrate from the anus. The proglottids of *T. solium* are actively motile in the stool, so they would appear to be capable of migrating from the anus as well. Since these proglottids contain infective eggs and cannot be identified to the species level at first glance, it is very important that personnel use precautions to prevent accidental infection with *T. solium* eggs, which may lead to cysticercosis. It is also very important that all patients be instructed to use good personal hygiene if they are found to have *Taenia* tapeworm infection, since there may be a time lag between diagnosis and initiation of therapy and a danger of autoinfection with the eggs or possible infection of others.

Using DNA differential diagnosis by base excision sequence scanning thymine-base reader analysis with mitochondrial genes, four distinct *Taenia* species can be identified: *T. saginata*, *T. asiatica*, and two genotypes of *T. solium*. Thus, if nucleotide databases are available, this system provides a useful tool for the identification of taeniid specimens from patients and for the study of genetic variations of these helminths (29).

Coproantigen Detection. Preliminary enzyme-linked immunosorbent assay (ELISA) studies for the diagnostic detection of *T. solium* and *T. saginata* coproantigens appear to be very promising (30–32). The ability to detect human taeniasis by coproantigen ELISA in the absence of eggs in the stool would represent a significant advance in diagnosis. Also, the ability to reliably identify *Taenia* carriers in areas where infections are endemic would improve epidemiologic studies. High concentrations of coproantigens are present until approximately 6 to 17 days after treatment. In cases of treatment failure, the ELISA was positive after day 15 of follow-up. For cured patients, ELISA results became negative at day 30 (33).

Tapeworm carriers are a source of infection for themselves, their households, and their villages. Rapid confirmation of cure (or treatment failure) is important. To allow months for the detection of treatment failure increases the likelihood these patients will be lost to follow-up, and thus, they may again become sources of infection. Cure of the tapeworm carriers, the only source of infection, is critical for the control of this zoonotic infection. The ELISA is a useful technique for early detection of treatment success or failure. However, as of 2015, there is no commercial source for this test.

Cysticercosis. Cysticercosis presents with a wide spectrum of morphological patterns ranging from viable cysts, cuticle fragments, scolex, and parenchyma to necrotic and calcified lesions. The diagnosis of cysticercosis usually depends on surgical removal of the parasite and microscopic examination for the presence of suckers and hooks on the scolex. Multiple larvae are frequently present, and the presence of cysticerci in the subcutaneous or muscle tissues indicates that the brain is probably also involved. The calcified larvae can be readily seen on X-ray examination (Fig. 16.19). CT scans or MRI may reveal the presence of lesions in the brain (Fig. 16.20). Although neurocysticercosis may be suspected, particularly in a patient living in an area of endemicity, the multiple neurologic syndromes and the various clinical presentations make it difficult to confirm the diagnosis, even with CT or MRI techniques. The primary consideration may be brain tumor. If the racemose form is present in the brain, the CT scan cannot distinguish the lesion from that caused by other tumors. Ultrafast CT has been used successfully to diagnose a case of cysticercosis involving the myocardium (34). Ocular cysticercosis can usually be diagnosed by visual identification of the movements and morphology of the larval worm.

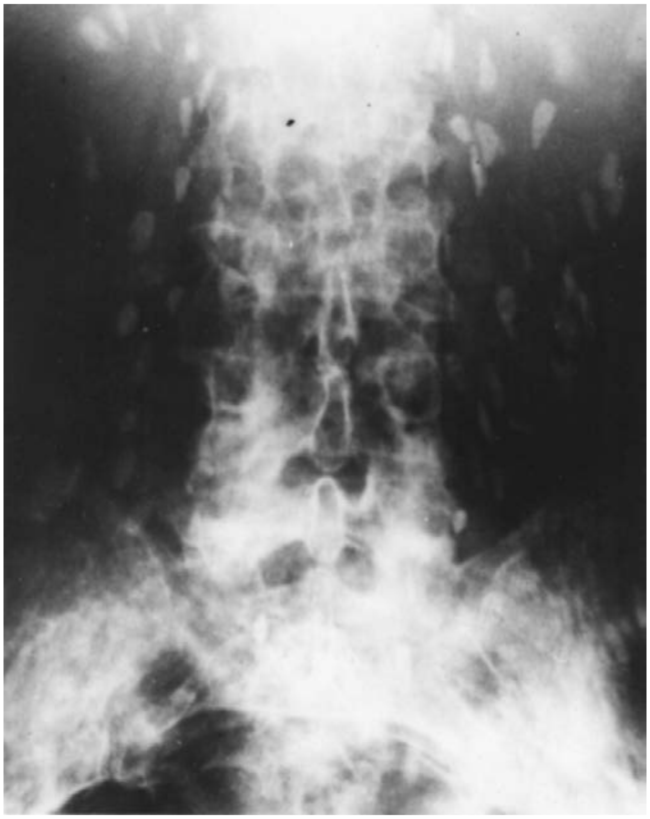


Figure 16.19 Calcified cysticerci in tissue. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch16.f19

Fine-needle aspiration cytology (FNAC) has also been recommended as a cost-effective procedure, since it eliminates the need for open biopsy. The characteristic morphology of parasitic tegument and parenchyma has proven to be very helpful. The cytological diagnosis is definitive in cases where actual parasite structure is identified in the smears. The viable cyst and the necrotic and calcified lesions all have distinctive morphological patterns. The viable cyst yields clear fluid and shows fragments of bladder wall in a clear acellular background. There is little or no inflammatory response. Single and detached hooklets may be the only recognizable remnants in aspirates of calcified cysts. They can often be visualized using routine and/or special stains (Giemsa, Ryan's modified trichrome) (35) (Fig. 16.21). Aspirates of necrotic and degenerated lesions may contain fragments of bladder wall, including calcareous corpuscles, detached single hooklets, and an infiltration of inflammatory cells, associated with the development of foreign body granulomas. The presence of eosinophils, neutrophils, palisading histiocytes, giant cells, and a typical granular dirty background in an aspirate from subcutaneous nodule

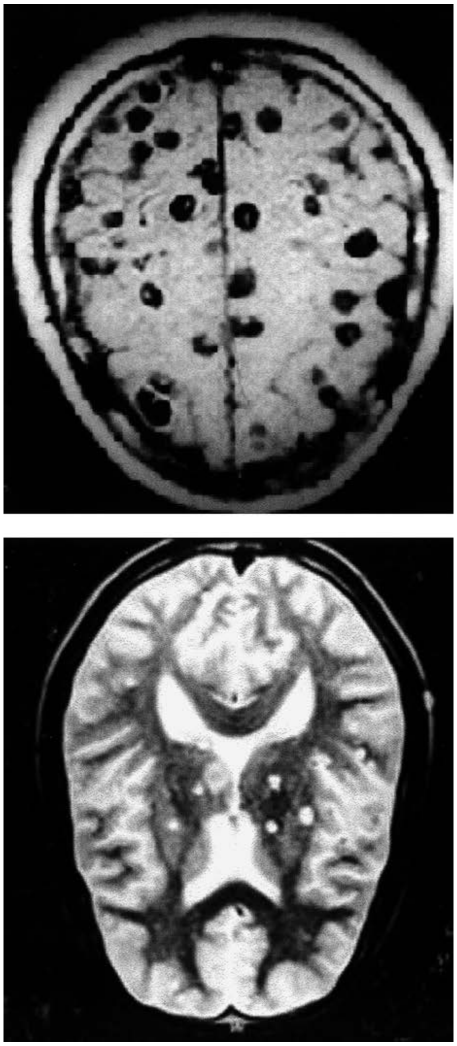


Figure 16.20 Neurocysticercosis. Scanning images of cysticerci in brain. (Upper, adapted from the Public Health Image Library, Centers for Disease Control and Prevention; lower, from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch16.f20

should alert the pathologist to a possible parasitic infestation. Nonetheless, in some cases of cysticercosis, none of these features may be present, and the inflammatory infiltrate may also be variable.

Review of FNAC smears from 258 patients with cysticercosis showed that Charcot-Leyden crystals were conspicuous by their absence, although cholesterol crystals were often seen (36). Absence of Charcot-Leyden crystals in cysticercosis could be attributed to the high lipid content of these lesions, as indicated by the frequent occurrence of cholesterol crystals.

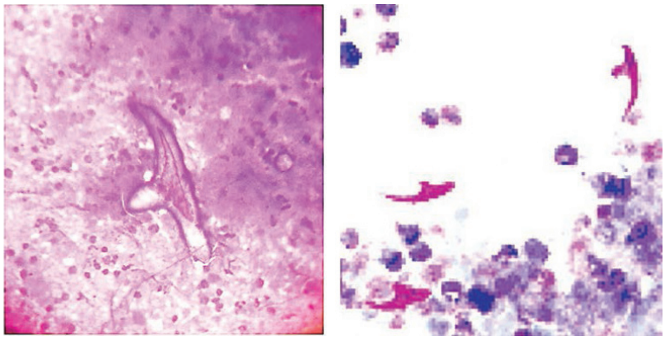


Figure 16.21 Tapeworm hooklets. (Left) Cysticercosis, hooklet seen on FNAC (Giemsa stain) (courtesy of Sawhney M, Agarwal S, reference 35). (Right) *Echinococcus granulosus* hooklets (Ryan's modified trichrome stain) (courtesy of Clavel A et al, *J Clin Microbiol* **37**:1561–1563, 1999).
doi:10.1128/9781555819002.ch16.f21

Cysticercosis Serologic Testing. The serodiagnostic methods currently available have variable sensitivity and specificity depending upon the clinical presentation, course of the disease, and diagnostic test methods. Questions concerning accurate serological screening tools for human cysticercosis are frequently raised. However, after symptomatic therapy, patient management is driven by the characteristics of the central nervous system infection (viability, number, location size and evolutionary stage of parasites, and the resulting inflammation). In the absence of neuroimaging, it is somewhat unclear whether serological confirmation of suspected symptomatic cases or detection of asymptomatic cases in population screening would influence patient management or prognosis (37).

The immunoblot assay for neurocysticercosis has been a definite improvement in antibody detection and is available at the Centers for Disease Control and Prevention (38, 39). A semipurified extract of *T. solium* cysticerci is used as the antigen, with seven major glycoprotein bands (50 to 13 kDa); a serum reaction with any one of these bands is indicative of cysticercosis. The sensitivity is 98% and the specificity is 100% (39). Both a single parietal cyst and subcutaneous infections without neurologic involvement are usually detected by this procedure. This test is more sensitive than the ELISA procedure, especially when using serum (40). In another study using CSF, the ELISA results were positive for 22 of 24 patients with active neurocysticercosis; however, six cases with calcified cysts and one case in transition were both negative. Thus, a significant difference was seen between active and inactive neurocysticercosis (41).

An enzyme-linked immunoelectrotransfer blot (EITB) assay using blood dried on filter paper has proven to be a convenient, economical way of handling field samples for epidemiologic surveys of cysticercosis, particularly in underdeveloped countries where refrigeration and venipuncture represent problems with specimen collection and handling. There is no decrease in sensitivity compared with traditional serum samples used in the EITB assay (42).

Using the EITB assay on CSF samples, the sensitivity of one study was 86% (43). Patients with hydrocephalus had higher levels of circulating antigen, and there was no difference between antigen levels in CSF taken before and 14 days after therapy. There was a high correlation between the number of live cysts detected by CT and the number and intensity of antibody reactions. In contrast, there was a negative correlation with the number of enhancing lesions seen using CT, supporting the hypothesis that enhancing lesions correspond to the dead stage of the parasite.

The serodiagnostic assay recognized by the World Health Organization and the Pan American Health Organization for cysticercosis and neurocysticercosis uses lentil lectin-purified glycoproteins (LLGP) in an EITB format. Although this assay has high sensitivity and specificity, antigen purification requires equipment and personnel not available in many areas where the disease is endemic. In an attempt to develop an automated diagnostic test for cysticercosis with high sensitivity and specificity, the QuickELISA format has been used. QuickELISA (Immunetics, Inc., Boston, MA) is a quantitative assay and can be automated for high throughput in field studies. Although this approach can be performed manually on the laboratory bench top, it can also be fully automated (44). Using the T24H recombinant antigen, the sensitivity and specificity of the QuickELISA were 96 and 99% for serum samples from persons infected with two or more viable cysts. These values are comparable to the sensitivity of 98% and specificity of 100% for the EITB in persons with multiple, viable cysts. Thus, the use of recombinant and synthetic diagnostic antigens in QuickELISA increases test specificity, eliminates the need for purification of antigens from parasitic material, and subsequently decreases the manufacturing cost of detection assays.

KEY POINTS—LABORATORY DIAGNOSIS

Taenia solium

1. Preliminary examination of the proglottid may not allow identification without clearing or injection of the uterine branches with India ink.
   Note Because of the possible danger of egg ingestion, specimens and proglottids should be handled with extreme care. The proglottid uterine structure will be packed with viable, infectious eggs, even if the proglottids are received in fixative.
2. The eggs look identical to those of *T. saginata*, and no identification to the species level can be performed with eggs alone without special procedures.
3. *Any patient with* Taenia *eggs recovered in the stool should be cautioned to use good hygiene. These patients should be treated as soon as possible to avoid the potential danger of accidental infection with the eggs, which may lead to cysticercosis (*T. solium*).*
4. Although serologic reagents for antibody detection and antigen detection tests have been developed, not all reagents produce sensitive and specific results. Consultation with the Centers for Disease Control and Prevention and/or your state public health laboratories may be helpful.

Treatment

Adult Worm. The use of praziquantel or niclosamide has been recommended (see chapter 36). Since there is always the possibility of cysticercosis via autoinfection, patients should be treated as soon as they are diagnosed.

Cysticercosis. Surgical removal is recommended when possible. In patients with ocular cysticercosis, cyst removal rather than enucleation is recommended. To prevent loss of the eye, it is recommended that the cysticercus be removed while it is still living; antiparasitic drugs may lead to a loss of vision secondary to intraocular inflammatory reaction. Excisional biopsy is recommended for subconjunctival cysticercosis. Idiopathic cystic myositis can present like extraocular muscle cysticercosis but can be differentiated by resolution with corticosteroid treatment. Therapy for orbital cysticercosis with oral albendazole and corticosteroids can prevent recurrent inflammation and improve ocular mobility (45).

The introduction of new drugs for cysticercosis has significantly changed the approach to patient management. Two drugs, praziquantel and albendazole, are effective against the cysticerci (46). Praziquantel is rapidly absorbed and reaches its peak concentration in plasma in 1.5 to 2 h; it can cross the blood-brain barrier, and the concentration in CSF is approximately 24% of that obtained in the plasma (46). Apparently, the drug action damages the worm tegument to produce spastic paralysis of the scolex. Albendazole is a broad-spectrum anthelmintic drug; the anticysticercal impact is by inhibition of glucose uptake by parasitic membranes. In a study of 114 patients with parenchymal brain cysticercosis, albendazole was found to be more effective than praziquantel for both a full and a short course of therapy. Also, the length of albendazole therapy could be shortened without loss of efficacy; in contrast, a short course of praziquantel caused a 12% reduction in drug effectiveness. From the results of this study, an 8-day course of albendazole is recommended as treatment for parenchymal brain cysticercosis; a 15-day course of praziquantel could subsequently be used in patients demonstrating partial response to albendazole (47). The issue of medical or surgical therapy for subarachnoid or ventricular cysticercosis is controversial; however, successful therapy with albendazole has been reported for a patient with cysticerci in both subarachnoid and ventricular locations (48).

The prognosis for the patient is excellent when the adult worm is present, good when the cysticerci can be surgically removed or medically treated, and poor when the racemose form of the parasite is present, particularly in the brain. However, treatment of hydrocephalus secondary to cerebral racemose cysticercosis using endoscopic third ventriculostomy appears to be an effective and safe technique; the efficacy of endoscopic third ventriculostomy will be evaluated in additional studies (49).

The goal of anticysticercal therapy is the simultaneous destruction of multiple cysts, then controlling the resulting inflammatory reaction with steroids. Most comparative investigations have shown that albendazole is more effective than praziquantel in reducing the number of cysts and in inducing overall clinical improvement with fewer adverse reactions. However, most of these trials have been uncontrolled (50).

Epidemiology and Prevention

Although other animals occasionally harbor the cysticercus stage, poorly cooked or raw pork is the usual source of human infection for the development of the adult worm. Three cases of cysticercosis in black bears in three northern California counties have been confirmed as due to *T. solium* (51). The number and geographic separation of the cases indicate that infection was not due to a single contaminated source but to human fecal material contaminated with *T. solium* eggs. How much of a threat this human-to-bear transmission poses to the bear population and/or to hunters (cysticercosis) is unknown, as is the reverse situation, bear-to-human transmission (adult worm). Human infection with cysticerci can involve thousands of organisms obtained from various sources: ingestion of *T. solium* eggs in contaminated food or water; self-infection from the presence of the adult worm in the intestine; and possibly internal autoinfection, in which the eggs come in contact with the stomach acid, thus possibly allowing hatching and penetration of the larvae into the tissues.

Prevention involves awareness of the infection route and the use of good sanitary and personal hygiene measures. The ingestion of vegetables fertilized with sewage should also be avoided. Adequate requirements for human sewage disposal should be followed. One of the most important means of prevention is the adequate cooking of pork and pork products; thorough cooking at 65°C kills the cysticerci; however, salting or pickling of the meat is usually not effective. In pig carcasses held at 4°C, more than 80% of the cysticerci were viable between days 1 and 25 (52). These findings confirm that eating raw or undercooked pork or viscera of pigs following refrigeration at 4°C for less than 30 days can lead to infection with *T. solium*. Effective immune protection of pigs against cysticercosis through vaccination has also been reported (53). The use of the EITB assay in sentinel piglets has been used to determine environmental contamination with *T. solium* eggs. This approach permits an indirect assessment of human risk and could be useful in monitoring the efficacy of intervention programs.

For a number of reasons, *T. solium* is a candidate for control: (i) neurocysticercosis has a tremendous impact on human health; (ii) since *T. solium* is the only source of cysticercosis for both humans and pigs, it may be epidemiologically controllable; (iii) effective and practical therapeutic intervention is available; (iv) international commitment to the use of control measures is present; and (v) the cost is acceptable. Through education, allocation of funds, recognition of both the human and veterinary issues, implementation of control measures and chemotherapy, morbidity and mortality may be drastically reduced throughout the world (53).

Taenia saginata

T. saginata was apparently differentiated from *T. solium* in the late 1700s; however, cattle were not identified as the intermediate host until 1863. This infection has a worldwide distribution and is generally much more common than *T. solium* infection, particularly in the United States (Table 16.1). The overall impact on human health is much smaller than that seen with *T. solium*, since cysticercosis with *T. saginata* is apparently quite rare.

Life Cycle and Morphology

The life cycle is very similar to that of *T. solium* (Fig. 16.22). Infection with the adult worm is initiated by the ingestion of raw or poorly cooked beef containing encysted *T. saginata* larvae. As with *T. solium*, the larva is digested out of the meat in the stomach, and the tapeworm evaginates in the upper small intestine and attaches to the intestinal mucosa, where the adult worm matures within 5 to 12 weeks. The adult worm can reach 25 m but often measures only about half this length. Although a single worm is usually found, multiple worms can be present.

The scolex is "unarmed" and has four suckers with no hooks (Fig. 16.23). The proglottids usually number 1,000 to 2,000, with the mature proglottids being broader than long and the gravid proglottids being narrower and longer. Identification to the species level is usually based on the number of main uterine lateral branches, which are counted on one side of the gravid proglottid. There are between 15 and 20 branches, with an average of 18 (Fig. 16.24).

Gravid proglottids often crawl from the anus during the day, when the host is most active. These proglottids

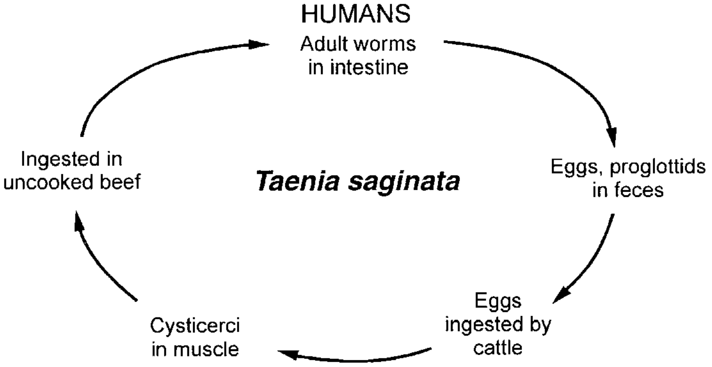
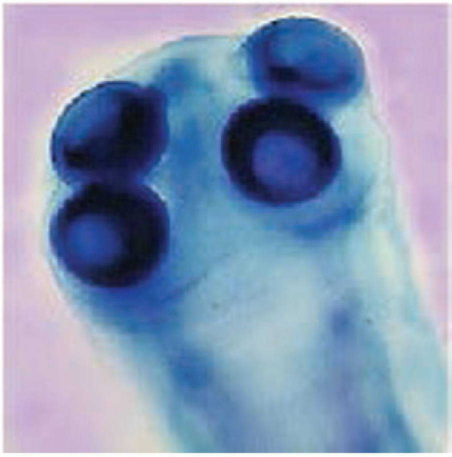


Figure 16.22 Life cycle of *Taenia saginata*. The same life cycle is applicable to *T. asiatica* (except that the eggs are contained primarily in the liver of the pig). doi:10.1128/9781555819002.ch16.f22

may crawl under the stool when a specimen is submitted unpreserved in the stool collection carton. The eggs cannot be distinguished from those of *T. solium*. They are round to slightly oval, measure 31 to 43 µm, have a thick, striated shell, and contain the six-hooked embryo (oncosphere) (Fig. 16.12 and 16.13). The eggs can remain viable in the soil for days to weeks.

On ingestion by cattle, the oncospheres hatch in the duodenum, penetrate the intestinal wall, and are carried via the lymphatics or bloodstream, where they are filtered out in the striated muscle. They then develop into the bladder worm or cysticercus within approximately 70 days. The mature cysticercus measures 7.5 to 10 mm wide by 4 to 6 mm long and contains the immature scolex, which has no hooks (unarmed). Other animals found to harbor cysticerci include the buffalo, giraffe, llama, and possibly reindeer (8). Actual cases of human cysticercosis with *T. saginata* are rare in the literature, and there is speculation that some reported cases have been inaccurately diagnosed.

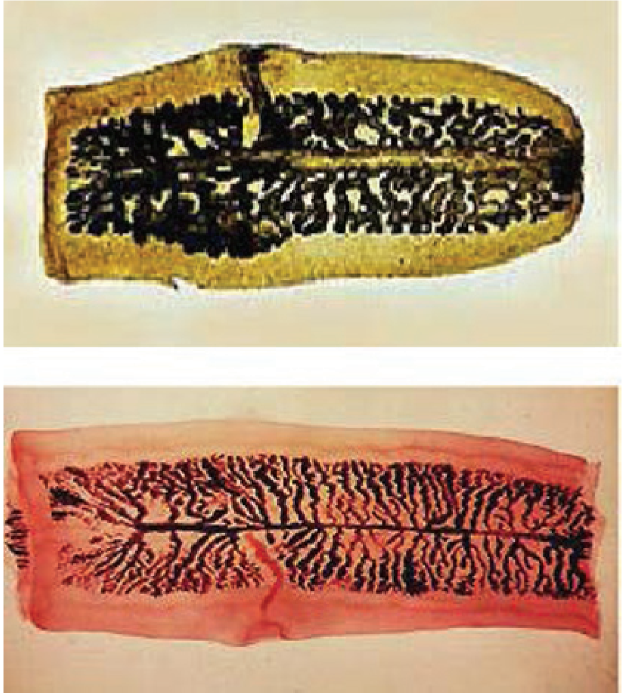
Figure 16.23 Scolex of *Taenia saginata*. doi:10.1128/9781555819002.ch16.f23



Clinical Disease

There are usually few symptoms associated with the presence of the adult worm in the intestine. Although rare symptoms (obstruction, diarrhea, hunger pains, weight loss, and appendicitis) have been reported, the most common complaint is the discomfort and embarrassment caused by the proglottids crawling from the anus. This occurrence may be the first clue that the patient has a tapeworm infection. Occasionally, the proglottids are also seen on the surface of the stool after it is passed.

Figure 16.24 Gravid proglottids of *Taenia saginata*. (Upper) India ink injection. (Lower) India ink injection dehydrated in xylenes containing some Wheatley's trichrome stain (provides background color). doi:10.1128/9781555819002.ch16.f24



Diagnosis

Since the eggs of *T. saginata* and *T. solium* look identical, identification to the species level is normally based on the recovery and examination of gravid proglottids, in which the main uterine lateral branches are counted (15 to 20 for *T. saginata* and 7 to 13 for *T. solium*). Often, the gravid proglottids of *T. saginata* are somewhat larger than those of *T. solium*; however, this difference may be minimal or impossible to detect when looking at fresh or preserved proglottids. If the scolex is recovered after therapy (which may require purgation), there will be four suckers and no hooks (Fig. 16.23).

KEY POINTS—LABORATORY DIAGNOSIS

Taenia saginata

1. Preliminary examination of the gravid proglottid may not allow identification without clearing or injection of the uterine branches with India ink.
   Note Since there is always the possible danger that the proglottid is *T. solium*, with the inherent problem of egg ingestion and cysticercosis, all specimens should be handled with extreme caution. In the case of *T. solium*, the proglottid uterine structure will be packed with viable, infectious eggs, even if the proglottids are received in fixative.
2. The eggs look identical to those of *T. solium*, so identification to the species level cannot be performed with eggs alone without special procedures.
3. Although gravid proglottids of *T. saginata* are often somewhat larger than those of *T. solium*, this difference may be very minimal or impossible to detect.
4. *Any patient with* Taenia *eggs recovered in the stool should be cautioned to use good hygiene. These patients should be treated as soon as possible to avoid the potential danger of accidental infection with the eggs, which may lead to cysticercosis (*T. solium*).*

Treatment

The use of praziquantel or niclosamide has been recommended (see chapter 36). The same general approach used for *T. solium* is used to eradicate the adult worm. Therapy is usually very effective; however, if the proglottids begin to reappear in the stool or crawl from the anus, retreatment is necessary.

Epidemiology and Prevention

Since human infection is acquired from the ingestion of raw or poorly cooked beef, all beef prepared for human consumption should be inspected for the presence of cysticerci. Thorough cooking of beef provides complete protection. A reliable method to detect infected cattle is needed, particularly because of public health concerns. The method of choice for routine serologic surveillance of cysticercosis is probably ELISA; however, previous antigenic reagents have lacked the necessary sensitivity and/or specificity. To improve the sensitivity and specificity of an existing IgM monoclonal antibody (MAb)-based ELISA for the detection of circulating antigen in the sera of cattle infected with *T. saginata* metacestodes, a modified sandwich ELISA was developed. When the original IgM MAb-based ELISA was compared with the IgG MAb-based ELISA using heat-treated sera from animals harboring more than 50 living metacestodes of *T. saginata*, the sensitivity increased from 56% with the former assay to 92% with the latter. Only a few animals carrying fewer than 50 cysts were detected by both the ELISA using IgG MAbs and that using IgM MAbs. The specificity of the IgM and IgG MAb-based ELISAs was 93.4 and 98.7%, respectively (54). To minimize the chance of infection, cattle should not be allowed to graze on ground contaminated by human sewage. Egg viability can range from a couple of weeks in untreated sewage to approximately 5 months on grass. According to Beaver et al. (8), the rate of bovine cysticercosis in the United States reached 0.37% in 1930 and has remained around this level in federally inspected cattle.

Taenia asiatica (Asian *Taenia* or *Taenia saginata asiatica*)

Recent epidemiologic studies of taeniasis in Southeast Asia indicate the presence of a third form of human *Taenia* sp. which can be distinguished from *T. saginata* and *T. solium*. This newly recognized cestode was originally called the Taiwan *Taenia* sp. and was first detected in Taiwanese aboriginals. However, it is now referred to as the Asian *Taenia* sp. or *T. asiatica* since it has also been found in a number of other Asian countries, including China, Indonesia, Japan, Korea, the Philippines, Thailand, and Vietnam. So far, it has not been reported in Africa, Europe, or America. Genetic studies have been used to determine whether the Asian *Taenia* sp. should be considered a new species or a subspecies, strain, or variant of *T. saginata*, which it closely resembles. The use of PCR-restriction fragment length polymorphism determined the cestode to be different from either *T. solium* or *T. saginata*. However, mitochondrial and nuclear sequence comparisons indicate that the Asian *Taenia* sp. is more closely related to *T. saginata*. These results support earlier conclusions that the Asian *Taenia* sp. is a genetically distinct entity but is closely related to *T. saginata*. Although there is no total agreement, its taxonomic classification as a subspecies or strain of *T. saginata* may be changed to a new species designation (55, 56). Because of this close relationship with *T. saginata*, any public health significance would be unlikely, since human cysticercosis is caused almost exclusively by *T. solium*.

Life Cycle and Morphology

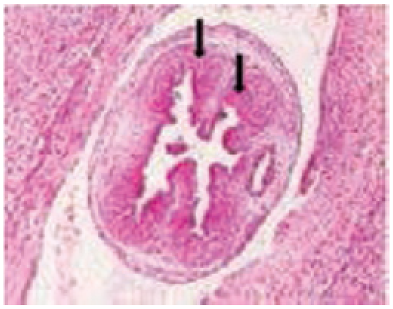
The life cycle is similar to that of *T. saginata*. However, cysticerci of *T. asiatica* develop in the liver of pigs, cattle, and goats, rather than the muscles of cattle. The cysticerci are much smaller than those of *T. saginata* or *T. solium* and measure approximately 0.5 to 1.7 mm by 0.5 to 2.0 mm. The protoscolex is armed, but the hooklets are lost in the mature worm (Fig. 16.25). The adult worm matures in 10 to 12 weeks, like *T. saginata*, but is smaller, measuring 4 to 8 m long, and contains 300 to 1,000 proglottids (Fig. 16.26). In some indigenous populations in Taiwan, 10 to 20% of the people are infected, primarily from the ingestion of pork meat and viscera. Another difference appears to be the presence of multiple worms, whereas infections with *T. saginata* are usually limited to a single worm. In one area of Taiwan during the late 1990s, the rate of *T. asiatica* infection was 11% and the annual economic loss was calculated at US$18,673,495. These and other figures suggest that taeniasis not only is a significant public health problem, but also leads to severe economic losses in East Asia.

Clinical Disease

Infected individuals may not be aware of the infection and may remain asymptomatic. However, they may complain of having abdominal pain, nausea, weakness, weight loss, changes in appetite, and headache. These symptoms suggest that the tapeworm causes irritation; biopsies of the mucosa may reveal inflammatory changes. Eosinophilia is seen in some patients.

It has been stated that *T. asiatica* does not cause human cysticercosis, since its molecular makeup is so similar to *T. saginata*. This could be related to the different composition of digestive enzymes or bile acids among different intermediate hosts. Also, *in vitro* hatching features differ significantly between *T. saginata* and *T. asiatica* eggs. However, the question of whether *T. asiatica* can cause cysticercosis in humans remains to be definitively answered. It has been suggested that liver ultrasonography be performed to confirm the presence of cysticerci in patients harboring *T. saginata*-like adults. Just as in pigs, only the molecular analysis of the extracted cysticerci could identify the species causing human cysticercosis (57).

Figure 16.25 *Taenia saginata asiatica* (*Taenia asiatica*). Cross section of cysticercus (photograph courtesy of Ping-Chin Fan, with permission). Note the suckers (arrows).
doi:10.1128/9781555819002.ch16.f25



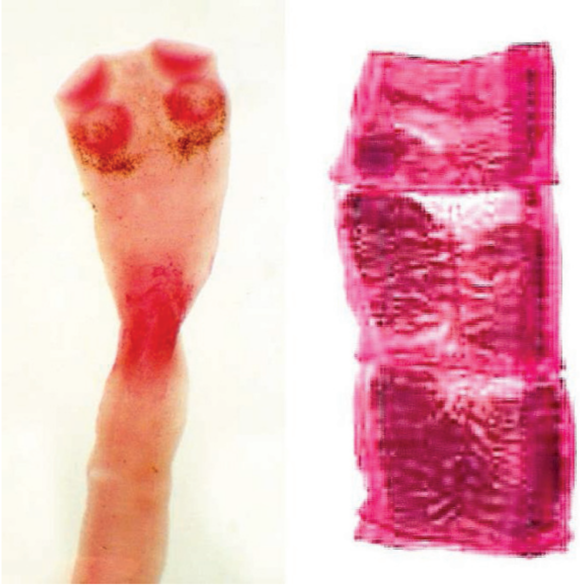


Figure 16.26 *Taenia asiatica* (*T. saginata asiatica*). (Left) Scolex from adult worm (unarmed, no hooks). (Right) Gravid proglottid. (Photographs courtesy of Ping-Chin Fan, with permission.)
doi:10.1128/9781555819002.ch16.f26

Diagnosis

T. asiatica has been misdiagnosed as *T. saginata* for more than 200 years in Asia and the same might currently occur in the rest of the world. Since the morphologies of *T. asiatica* and *T. saginata* are so similar, the diagnosis may rely on the patient's history. Have they ingested undercooked or raw beef (*T. saginata*) or raw pig liver (*T. asiatica*)? Because the morphology of the two is so similar, it has been suggested that the disease be called *T. saginata/asiatica* taeniasis.

With no known specific immunological test for *T. asiatica*, only the molecular analysis of the extracted cysticerci can be used to confirm the species causing pig cysticercosis. Therefore, *T. asiatica* may be misdiagnosed as *T. solium* not only in humans but also in pigs. Any pig cysticercosis around the world diagnosed only by immunological methods could be produced by *T. asiatica*, so it has been suggested that cysticercosis be called *T. solium/asiatica* cysticercosis.

Treatment

The use of praziquantel or niclosamide has been recommended (see chapter 36). The same general approach used for *T. solium* and *T. saginata* is used to eradicate the adult *T. asiatica* worm. Therapy is usually very effective; however, if the proglottids begin to reappear in the stool or crawl from the anus, retreatment is necessary.

Treatment for cysticercosis would be the same as for *T. solium* (above). Again, without molecular testing of the actual cysticercus, definitive identification of *T. asiatica* or *T. solium* would be questionable.

Epidemiology and Prevention

In areas where *Taenia* tapeworms are endemic in Korea, *T. saginata* is dominant over *T. solium*; however, pig consumption is considerably higher than beef consumption. By examination of data published between 1963 and 1999, the ratio of armed versus unarmed tapeworms in humans was estimated at approximately 1:5. The ratio of pig eaters versus cattle eaters, however, was approximately 5:1. This inconsistency was explained with the recently described *T. asiatica*, which infects humans through the eating of raw pig viscera, primarily liver. Considering that the scolex of *T. asiatica* is unarmed and the consumption of visceral organs of pigs is higher than beef consumption, this information provides a clearer understanding of *T. asiatica* infection together with coexisting *T. saginata* and *T. solium* infection in Korea (58). Prevention depends on a thorough understanding of the epidemiology of this infection.

Hymenolepis (*Rodentolepis*) *nana*

Hymenolepis nana has been called the dwarf tapeworm and has a worldwide distribution. The fact that an intermediate host is not required in the life cycle was determined in the late 1800s by Grassi and Rovelli (8). For this reason, *H. nana* has been considered to be the most common tapeworm throughout the world, and it is estimated that 50 million to 75 million people worldwide are infected. The infection is most commonly seen in children, although adults are also infected (Table 16.1). There has been some discussion regarding placing *H. nana* in the genus *Rodentolepis*; however, for this edition of the book, we will continue to use *Hymenolepis* (*Rodentolepis*) *nana*.

The presence of *Rodentolepis microstoma* (rodent tapeworm) in humans has been reported for the first time; the data came from a 3-year survey of gastrointestinal parasites in remote communities in the northwest of Western Australia (59). However, to date, there is little information available regarding other human infections with this parasite.

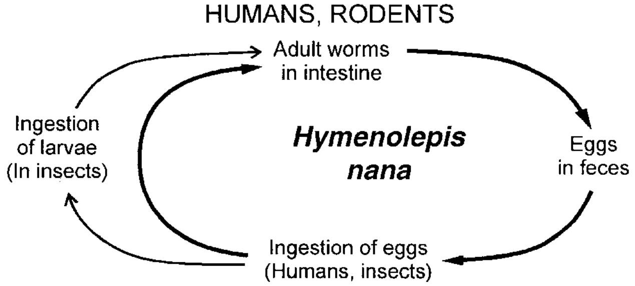


Figure 16.27 Life cycle of *Hymenolepis nana*. doi:10.1128/9781555819002.ch16.f27

Life Cycle and Morphology

The life cycle can be seen in Fig. 16.27. Infection is usually acquired by the ingestion of infective *H. nana* eggs, primarily from human stool (direct life cycle). The eggs hatch in the stomach or small intestine, and the liberated larvae, or oncospheres, penetrate the villi in the upper small intestine. The larvae develop into the cysticercoid stage in the tissue and migrate back into the lumen of the small intestine, where they attach to the mucosa (Fig. 16.28). The adult worms mature within several weeks. They are very small compared with worms of the *Taenia* species and measure up to 40 mm long. The more worms that are present, the shorter the total length of each worm. Insect intermediate hosts include grain- and flour-eating beetles such as *Tribolium* and *Tenebrio*; fleas such as *Pulex irritans*, *Xenopsylla cheopis*, and *Ctenocephalides canis*; and moths (Fig. 16.29). The indirect life cycle is initiated when the insect ingests an infective *H. nana* egg. Within the insect body cavity, the oncosphere contained within the egg becomes free of the egg membranes and transforms into the cysticercoid larval form, which is infective when the insect is ingested by the human host.

The scolex has four suckers and a retractable, prominent rostellum with hooks (Fig. 16.30). The adult worm is rarely seen in the stool. However, as the proglottids develop from immature to mature to gravid, they tend to be very wide and short (Fig. 16.30). The eggs are released by disintegration of the gravid proglottids, pass out in the stool, and are immediately infectious. They are round to oval with a thin shell and measure 30 to 47 μm in diameter. The oncosphere has two polar thickenings from which arise polar filaments that lie between the oncosphere and the shell (Fig. 16.31). Although accidental ingestion of the insect intermediate host can result in development of the adult worms, this mode of infection is probably rare. There is evidence that auto- or hyperinfection occurs when eggs spontaneously hatch in the small intestine and initiate a new life cycle. Although adult worms live for only about a year, some patients can carry a large worm burden for

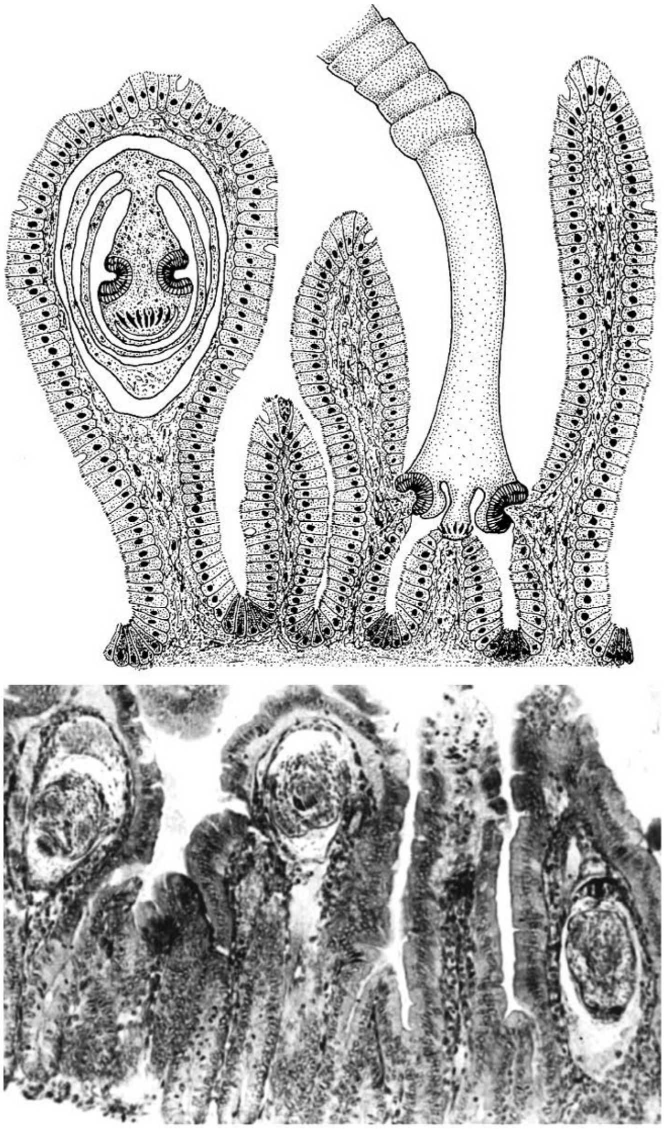


Figure 16.28 *Hymenolepis nana*. (Upper) Diagram of both a cysticercoid and adult worm in the small intestine. (Illustration by Sharon Belkin.) (Lower) Cysticercoids of *H. nana* in the villi of the small intestine. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch16.f28

many years, possibly due to the autoinfection capability within the life cycle or repeated reinfection.

Clinical Disease

Within the human host, the adult worms tend to be located in the upper portion of the ileum. An infection with *H. nana* may cause no symptoms, even with a heavy worm burden. Some patients complain of headache, dizziness, anorexia, abdominal pain, diarrhea, or possibly irritability, particularly if the worm burden is 1,000 to 2,000 worms. Some patients have a low-grade eosinophilia of 5% or more.

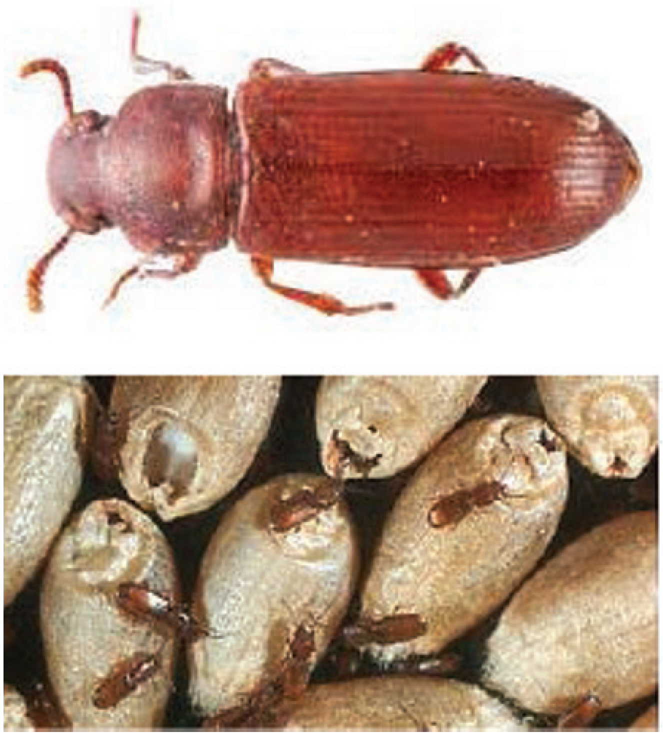
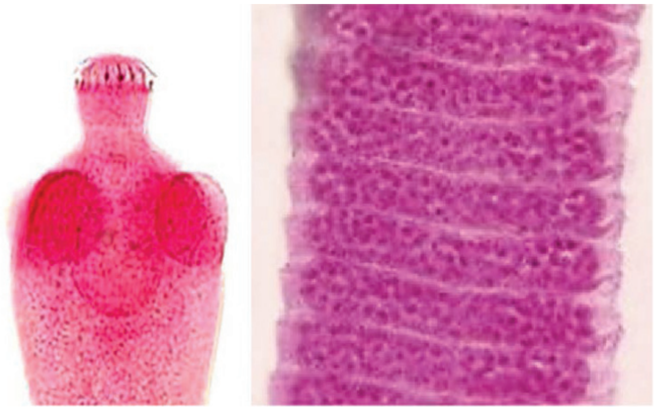


Figure 16.29 (Upper) *Tribolium confusum* (common pest found in stored grain products/flour), intermediate host of *Hymenolepis* spp. (about 4 mm long) (courtesy of CDC Public Health Image Library). (Lower) Grain beetles on wheat (courtesy of U.S. Department of Agriculture).
doi:10.1128/9781555819002.ch16.f29

Figure 16.30 *Hymenolepis nana*. (Left) Scolex; note the suckers and hooks on the rostellum. (Right) Proglottids; note they are very wide and short. The adult worms are rarely seen in human fecal specimens. (Courtesy of CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch16.f30



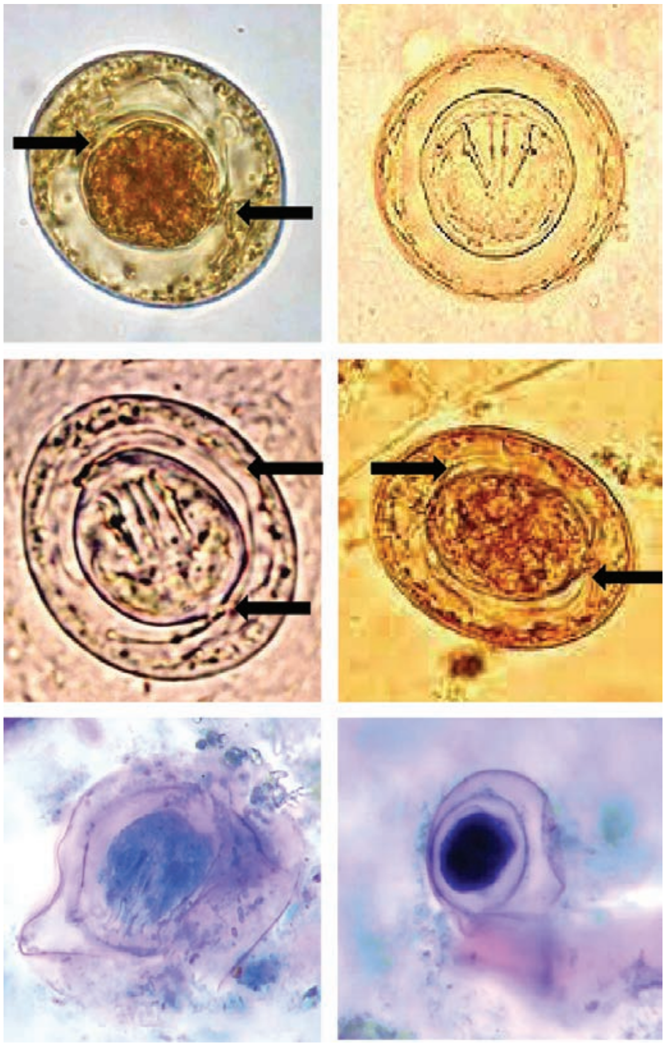


Figure 16.31 (Top, left) Egg of *H. nana*; note the thin egg shell, the six-hooked oncosphere, and the polar filaments that lie between the oncosphere and egg shell (arrows). (Top, right) Egg of *H. diminuta*; note the thin egg shell, the six-hooked oncosphere, and the lack of polar filaments. (Middle) *H. nana* eggs; note the polar filaments (arrows). (Bottom) Eggs of *H. nana* stained with Wheatley's trichrome stain; note the distorted appearance. doi:10.1128/9781555819002.ch16.f31

When young children have heavy infections, they may have loose stools or even diarrhea containing mucus. Persistent, diffuse abdominal pain seems to be the most common symptom. Heavy human infection may be attributed to internal autoinfection, in which the eggs hatch in the intestine and follow the normal life cycle to the adult worm.

Diagnosis

Since the adult worm or proglottids are rarely seen in the stool, the diagnosis is based on recovery and identification of the characteristic eggs containing polar filaments (Fig. 16.31). They are most easily seen and identified from fresh specimens or those preserved in fixatives that do not contain polyvinyl alcohol (PVA). Specimens preserved in PVA contain eggs that do not exhibit morphologic characteristics that are as well delineated as those seen in non-PVA-fixed specimens. These thin-shelled eggs also tend to collapse on the permanent stained smear and may be difficult to identify in this type of preparation; the direct wet film or concentration wet mount is recommended (Fig. 16.31).

KEY POINTS—LABORATORY DIAGNOSIS

Hymenolepis nana

1. Adult worms or proglottids are rarely seen in the stool.
2. Eggs with the characteristic thin shell, six-hooked oncosphere, and polar filaments are diagnostic.
3. Egg morphology is more easily seen in fresh specimens or those preserved in non-PVA-based fixatives.
4. The eggs are infectious for humans, and unpreserved stools should be handled with caution.

Treatment

Niclosamide has been widely used for years. However, the use of praziquantel in a single oral dose is apparently more effective and kills the cysticercoid as well as the adult worm. The recommended dose for adults and children is 25 mg/kg in a single dose (see chapter 36).

Epidemiology and Prevention

H. nana is the only human tapeworm in which an intermediate host is not necessary and transmission is from person to person. Children are usually infected more often than adults. Since infection is from person to person via the eggs, good personal hygiene is an important preventive measure. Infection from rats and mice is always a possibility, as is the accidental ingestion of infective insect intermediate hosts.

Hymenolepis diminuta

Although *H. diminuta* is commonly found in rats and mice, it is infrequently found in humans. It has a worldwide distribution in normal hosts, and fewer than 500 human cases, primarily from India, the former Soviet Union, Japan, Italy, and certain areas of the southern United States (Tennessee, Georgia, and Texas), have been reported (8, 60) (Table 16.1).

Life Cycle and Morphology

The life cycle is very similar to the indirect life cycle of *H. nana*, and the infection is considered a true zoonosis; the arthropod intermediate host is not optional but obligatory (Fig. 16.32). A number of different arthropods (lepidopterans, earwigs, myriapods, larval fleas, and various beetles) can serve as intermediate hosts. After egg ingestion by the

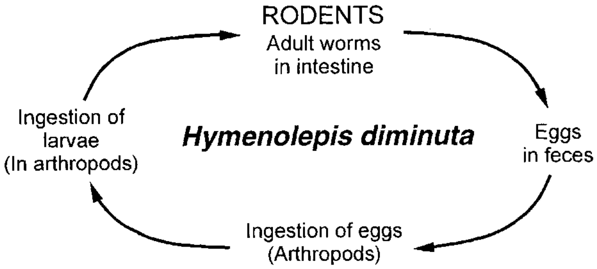
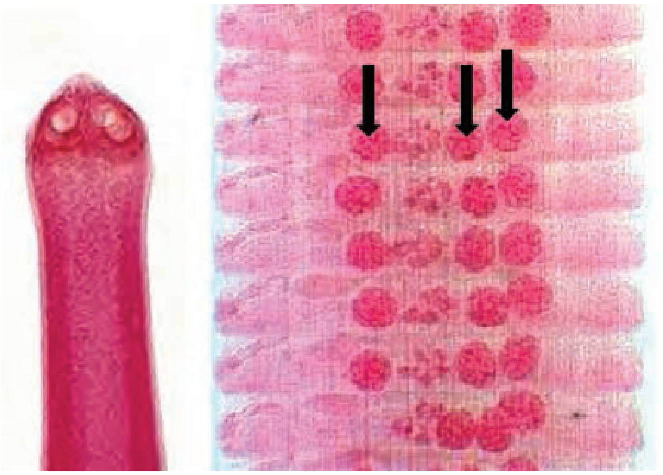

RODENTS
Adult worms in intestine
Eggs in feces
Ingestion of eggs (Arthropods)
Ingestion of larvae (In arthropods)
Hymenolepis diminuta

Figure 16.32 Life cycle of *Hymenolepis diminuta*. doi:10.1128/9781555819002.ch16.f32

arthropods, the cysticercoid stage forms, which is similar in morphology to the cysticercoid stage of *H. nana*. Infection by accidental ingestion of the infected arthropod containing cysticercoids results in development of the adult worm in the intestine of the human host. In contrast to *H. nana*, the scolex of *H. diminuta* has no hooks on the rostellum (Fig. 16.33). Multiple worms may be present, each being shorter than if a single worm were present. Although some of the adult worms (measuring 20 to 60 cm long) are occasionally spontaneously passed in the stool, usually the eggs are recovered and identified. They are morphologically similar to the eggs of *H. nana* and measure from 60 to 79 μm. They contain the six-hooked oncosphere and a clear area between the oncosphere and the shell. This area contains a gelatinous matrix that does not have polar filaments arising from the polar thickenings, as is seen in *H. nana* (Fig. 16.34).

Figure 16.33 *Hymenolepis diminuta*. (Left) Scolex; note the suckers and no hooks on the rostellum. (Right) Proglottids; note they are very wide and short. Also note the presence of the three oval testes (arrows). The adult worms are rarely seen in human fecal specimens. (Courtesy of CDC Public Health Image Library.) doi:10.1128/9781555819002.ch16.f33



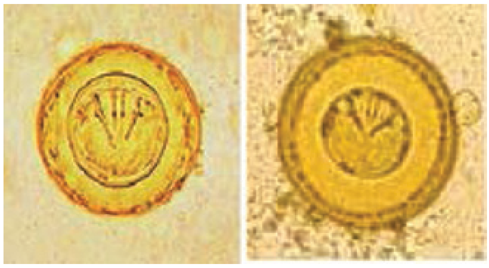


Figure 16.34 *Hymenolepis diminuta* eggs. Note the absence of polar filaments like those seen in *H. nana* eggs. doi:10.1128/9781555819002.ch16.f34

Clinical Disease

As with most tapeworms, the infection is usually tolerated very well by the host, with few if any symptoms. Most infections have been reported from children younger than 3 years; however, infected adults have also been reported. Symptoms that have been reported include diarrhea, anorexia, nausea, headache, and dizziness; they are most likely to be seen in infected children with a heavy infection.

Diagnosis

Since the proglottids usually disintegrate in the gut, diagnosis is based on recovery and identification of the characteristic eggs. The similarity to *H. nana* eggs is striking; however, there are no polar filaments in the eggs of *H. diminuta* (Fig. 16.34).

KEY POINTS—LABORATORY DIAGNOSIS

Hymenolepis diminuta

1. Although human infection is rare, diagnosis can be based on recovery and identification of the characteristic eggs (thin shell, presence of six-hooked oncosphere, and no polar filaments).
2. The eggs are occasionally submitted to laboratories for identification as a part of various proficiency testing programs. The important point is to be able to differentiate these eggs from those of *H. nana*.
3. Unlike *H. nana* eggs, the eggs of *H. diminuta* are not infectious from person to person.

Treatment

Praziquantel is recommended as a single dose of 5 to 10 mg/kg of body weight (see chapter 36).

Epidemiology and Prevention

Since the infection is acquired from accidental ingestion of the infected intermediate arthropod hosts, avoidance of this type of exposure is recommended. Possible situations include swallowing ectoparasites from the rodent host or accidentally ingesting beetles in precooked cereals. Rat control programs might also decrease the possibility of human exposure.

Dipylidium caninum

D. caninum is commonly found throughout the world in dogs and cats, both domestic and wild. Human infections have also been reported from many areas of the world, including the United States. Most reported infections have been in children (Table 16.1). A case reported in 2003 appears to be the first case reported in the American pathology literature during the last 36 years (MEDLINE database, 1966 to 2002) (61). Since then, approximately 10 additional cases have been reported in infants or small children.

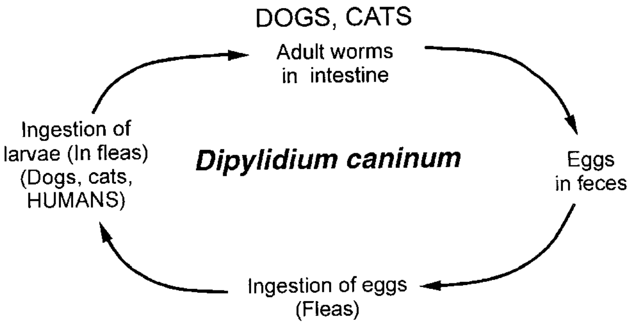
Life Cycle and Morphology

The life cycle is very similar to that of *H. diminuta*, in which the arthropod is an obligatory intermediate host (Fig. 16.35). The adult worms are found in the dog or cat intestine, and gravid proglottids separate from the strobila and may migrate singly or in short chains out of the anus. The adult worms measure 10 to 70 cm long and have a scolex with four suckers and an armed rostellum. The single proglottids have been described as looking like cucumber seeds when moist and like rice grains when dry (Fig. 16.36). Groups of eggs (egg packets) may be found in the stool (Fig. 16.37). Each egg measures from 25 to 40 µm and contains the six-hooked oncosphere. The eggs are ingested by the larval stages of the dog, cat, or human flea, in which they develop into cysticercoid larvae. When these fleas are then ingested by the definitive host (dogs, cats, or humans), the adult worm develops within 3 to 4 weeks.

Clinical Disease

The symptoms are related to worm burden; however, in most patients (usually children), complaints may consist of indigestion and appetite loss. The infection may first be noticed when proglottids migrate from the anus. Symptoms have been reported and can include abdominal pain, diarrhea, urticaria, and pruritus ani. A moderate eosinophilia may also be present.

Figure 16.35 Life cycle of *Dipylidium caninum*. doi:10.1128/9781555819002.ch16.f35


DOGS, CATS
Adult worms in intestine
Eggs in feces
Ingestion of eggs (Fleas)
Ingestion of larvae (In fleas) (Dogs, cats, HUMANS)
Dipylidium caninum

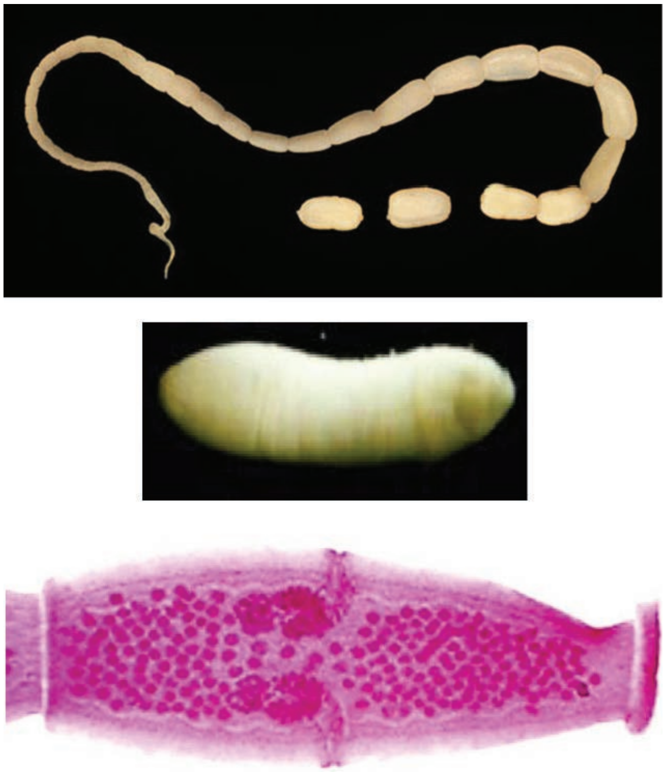


Figure 16.36 Proglottids of *Dipylidium caninum*. (Top) Fresh proglottid that may resemble a cucumber seed (note the shape and light color); when the proglottids dry, they may resemble rice grains. (Middle) Fresh, unstained proglottid. (Bottom) Stained proglottid. (Photographs from the Centers for Disease Control and Prevention Program: Laboratory Identification of Parasites of Public Health Concern.) doi:10.1128/9781555819002.ch16.f36

Diagnosis

Diagnosis depends on the recovery and identification of the characteristic egg packets or the proglottids. The proglottids appear like white cucumber seeds when fresh and rice grains when dry.

KEY POINTS—LABORATORY DIAGNOSIS

Dipylidium caninum

1. The egg packets are very characteristic and are frequently used to make the diagnosis. The individual eggs within the packet tend to have a thin shell and contain a six-hooked oncosphere.
2. Proglottids can be identified on the basis of shape (cucumber seeds when fresh, rice grains when dry) and the presence of egg packets that can be recovered from a ruptured proglottid.

Treatment

A single dose of praziquantel (10 mg/kg of body weight) for both adults and children is recommended (see chapter 36).

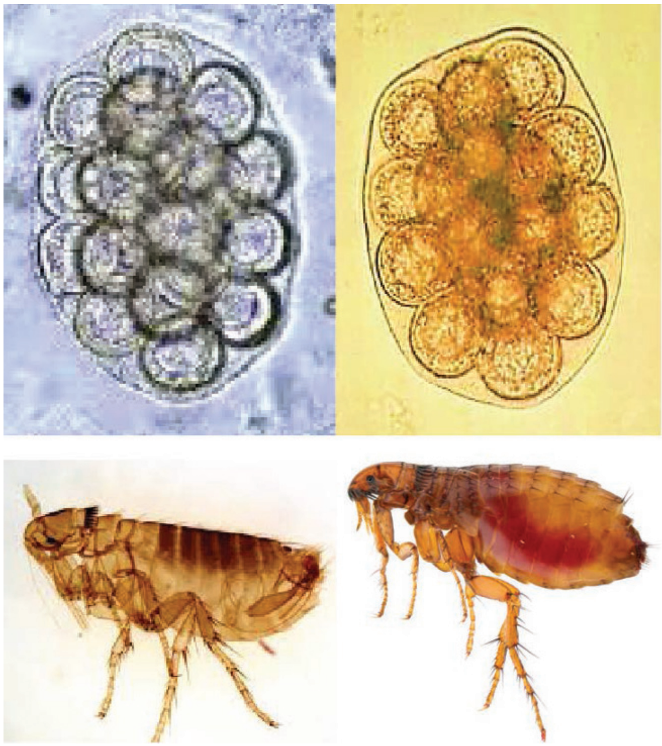


Figure 16.37 *Dipylidium caninum*. (Upper, left) Egg packet in a fecal wet mount; (right) egg packet in a wet mount with iodine. (Lower) Fleas that carry *D. caninum* (courtesy of CDC Public Health Image Library). doi:10.1128/9781555819002.ch16.f37

Epidemiology and Prevention

Most human cases have been in children, indicating that children may be more likely to accidentally swallow the infected fleas or may be more susceptible to infection. Periodic administration of anthelmintic agents to dogs and cats and use of flea powders will help reduce the risk of infection.

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17

Tissue Cestodes: Larval Forms

Echinococcus granulosus (cystic hydatid disease)

Echinococcus multilocularis (alveolar hydatid disease)

Echinococcus oligarthrus and *Echinococcus vogeli* (polycystic hydatid disease)

Taenia (*Multiceps*) spp. (*Taenia multiceps*, *Taenia serialis*) (coenurosis)

Spirometra mansonoides and *Diphyllobothrium* spp. (sparganosis)

NOTE *Taenia solium* (cysticercosis) is discussed in chapter 16.

Echinococcus granulosus (Cystic Hydatid Disease)

Currently, four species are recognized within the genus *Echinococcus: E. granulosus* (which causes cystic disease), *E. multilocularis* (which causes alveolar disease), *E. vogeli* (which causes polycystic disease), and *E. oligarthrus* (which causes polycystic disease) (Table 17.1). There is clear evidence of strain variation in *E. granulosus*, and 10 different strains have now been identified (Table 17.2) (1–9). *E. granulosus* is characterized by high intraspecific variability (genotypes G1 to G10), and according to the new molecular phylogeny of the genus *Echinococcus*, the *E. granulosus* complex has been divided into *E. granulosus* sensu stricto (G1 to G3), *E. equinus* (G4), *E. ortleppi* (G5), and *E. canadensis* (G6 to G10).

Based on historical information, hydatid cysts were recognized by Hippocrates, Aretaeus, and Galen. There was speculation concerning the possible relationship between human and animal hydatid cysts during the 1600s and 1700s. The adult worms and various aspects of the life cycle were studied in the late 1800s.

The areas of the world involved in sheep and cattle raising tend to be the areas where infections are endemic; they even include the Basque sheep farmers in California. The number of infections in both animals and humans has decreased over the years as a result of education and various control measures. However, figures suggest that the surgical incidence in some areas in Central Asia is now greater than 10 in 100,000 (up to 27:100,000 in Tadjikistan), and many of the cases are in children and the unemployed (10). The rates of infection in major livestock species such as sheep have also increased, with a doubling of prevalence, to a figure approaching 25% in some areas. There is also evidence to indicate a reemergence of canine *E. granulosus* infection in Wales. This has been associated with the large-scale outdoor slaughter of sheep during the 2001 foot-and-mouth disease outbreak in the United Kingdom and the suspected increased risk for transmission between sheep and dogs. The data suggest that allowing dogs to roam free and infrequent dosing of dogs with praziquantel have contributed to the increase; thus, increased transmission to humans appears probable in this setting (11).

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TABLE 17.1 Tissue cestodes

Characteristic	*Echinococcus granulosus*	*Echinococcus multilocularis*	*Echinococcus vogeli, E. oligarthrus*	*Taenia* (*Multiceps*) spp.	*Spirometra* and *Diphyllobothrium* spp.
Disease	Hydatid disease (cystic)	Hydatid disease (alveolar)	Neotropical echinococcosis; hydatid disease (polycystic); *E. oligarthrus* (unicystic)	Coenurosis	Sparganosis
Geographic location of the parasite	Worldwide	North America, northern and central Eurasia	Central and South America (85%) in Brazil, Colombia, Ecuador, Argentina	Worldwide	Worldwide; more common in China, Japan, Southeast Asia
Definitive host(s)	Domestic dog, wild canids (coyote, dingo, red fox, etc.)	Red fox, Arctic fox, raccoon dog, coyote, domestic dog, cat	Bush dog, domestic dog	Dogs, other canids	Dogs, cats
Acquired by	Egg ingestion (dogs)	Egg ingestion (foxes, cats)	Egg ingestion (dogs [*E. vogeli*], felids [*E. oligarthrus*])	Egg ingestion (dogs, other canids)	(i) Ingestion of infected *Cyclops* spp. (procercoid); (ii) ingestion of raw infected flesh of amphibians, reptiles, birds, mammals (spargana); (iii) local application of raw infected flesh as a poultice (spargana)
Intermediate host(s)	Primarily ungulates (sheep, cattle, swine, horses); also marsupials	Rodents (voles, lemmings, shrews, mice), other small mammals	Paca, agouti, spiny rat	Sheep, goats, cattle, horses, lagomorphs, rodents	Frogs, mammals
Stage of organism found in tissue	Larval form (fluid-filled unilocular hydatid cyst), contains protoscolices and daughter cysts, limiting membrane; 1–>15 cm; visceral, primarily liver and lungs	Larval form (alveolar hydatid cyst), no limiting membrane, usually sterile with no protoscolices; visceral, primarily liver	Larval form (fluid-filled polycystic hydatid cyst), scolex visible in wet mounts, large hooklets (38–46 μm long) and small hooklets (30–37 μm long); visceral, liver, abdomen, lungs (*E. vogeli*); orbits, heart (*E. oligarthrus*), vesicles partitioned by septa, protoscolices present	Larval form (intermediate between cysticercus and hydatid cyst)	(i) Procercoid; (ii) spargana; (iii) spargana (see above)
Type of growth in humans	Concentric expansion	Exogenous proliferation, tumorlike, similar to metastatic growth	Exogenous and endogenous proliferation (*E. vogeli*); expansive, no indication of exogenous proliferation (*E. oligarthrus*)	Multiple scolices, but no daughter cysts	More elongate, wormlike structure, no suckers or hooks; resemble narrow tapeworm proglottids, motile
Location in body	Liver (60%), lungs (20%), kidneys (4%), muscle (4%), spleen (3%), soft tissues (3%), brain (3%), bones (2%), other (1%)	All sites as for *E. granulosus*; most common site is liver, metastases in lungs, brain, bones, etc.	Liver, lungs (15%) (*E. vogeli*); eye and heart (*E. oligarthrus*)	Most often in central nervous system	Most tissues have been involved; depends on the site of the poultice application
Symptoms	Depends on cyst location, usually mechanical from enlarging cyst; may also be allergic reactions from cyst fluid leakage	Hepatic disease resembles slow growing mucoid carcinoma (no fever); hepato- and splenomegaly, jaundice, ascites	Hepatomegaly, palpable peritoneal masses, jaundice (*E. vogeli*)	Like space-occupying lesion in the central nervous system, similar to tumor, rarely muscles or subcutaneous tissues	Edema, pain, irritation, inflammation, toxemia, eye damage, elephantiasis if lymphatics involved, slowly growing, tender, subcutaneous nodules (may be migratory), ocular sparganosis
Treatment	Surgical removal, PAIR, albendazole, mebendazole less common	Albendazole, mebendazole; no role for praziquantel in these cases; surgery not recommended	Surgery plus albendazole; difficult to treat	Rarely diagnosed preoperatively; surgery; albendazole plus praziquantel	Surgical removal and drainage; drugs usually not effective, but triclabendazole can be used to supplement surgical removal; proliferating type: surgery almost impossible

TABLE 17.2 *Echinococcus granulosus* strains[a]

Strains (genotype, G)	Geographic distribution	Definitive host	Intermediate host	Infective for humans
G1: Common sheep strain	Europe, Middle East, Africa, Iran, India, Nepal, China, Russia, Australian mainland, Tasmania, New Zealand, United States, South America, Sardinia	Dog, fox, dingo, jackal, hyena	Sheep, cattle, pig, camel, goat, macropods, wild boar, red deer	Yes; *E. granulosus* (G1/G2/G3); responsible for majority of human cases (88%); most cosmopolitan distribution
G2: Tasmanian sheep strain	Tasmania, Argentina, Europe	Dog, fox	Sheep, cattle?	Yes
G3: Buffalo strain	Asia	Dog, fox?	Buffalo, cattle?	?
G4: Horse strain (*E. equinus*)	Europe, Middle East, South Africa (New Zealand?, United States?)	Dog	Horse, other equines	No
G5: Cattle strain (*E. ortleppi*)	Europe, South Africa, India, Nepal, Sri Lanka, Russia, South America?	Dog	Cattle, buffalo, sheep, goat, camel	Yes, rare
G6: Camel strain	Middle East, Iran, Africa, China, Nepal, Argentina	Dog	Camel, goat, cattle	Yes; *E. canadensis* (G6/G7, G8/G10); G6/G7 cause significant number of human infections (11%)
G7: Pig strain	Poland, Slovakia, Ukraine, Russia, Argentina, Europe, Sardinia	Dog	Pig, wild boars, red deer	Yes
G8: Cervid strain	North America, Eurasia	Wolf, dog	Cervids	Yes, rare
G9: Pig/human strain	Poland	?	?	? Existence of G9 as separate genotype remains controversial
G? (*E. felidis*)	Africa	Lion	Zebra, wildebeest, warthog, bushpig, buffalo, various antelope species, giraffe?, hippopotamus?	?
G10: Fennoscandian cervid strain	Finland	Wolf, dog	Cervids; closely related to G5, G6, and G7 strains	Yes, rare

[a] Adapted from references 1–9.

Life Cycle and Morphology

The life cycle of *E. granulosus* is presented in Fig. 17.1. The adult worms are very small (3 to 6 mm long) and consist of a scolex, a neck, and only a single proglottid at each stage of development (immature, mature, and gravid) (Fig. 17.2). There may be several hundred worms in the intestine of the canine host (usually the dog). The worms may survive in the host for up to 20 months, and each gravid proglottid contains few eggs (100 to 1,500), which are much larger than those of some of the other tapeworms. After the gravid proglottids and eggs are passed in the feces, they may be swallowed by an intermediate host, including humans. After exposure to gastric and intestinal enzymes, the eggs hatch in the duodenum. The released oncospheres penetrate the intestine and are carried via the bloodstream, where they are filtered out in the various organs. The most common site in humans is the liver (60 to 70% of cases). Although not all oncospheres survive this passage through the body, those that do survive begin to grow and develop. Usually by the fifth month, the wall of the hydatid cyst has become differentiated into an outer friable, laminated, nonnucleated layer and an inner nucleated germinal layer. Various daughter cysts (brood capsules) bud off from the inner germinal layer and may remain attached or float free in the interior of the fluid-filled cyst. The individual protoscolices bud off from the inner wall of the daughter cysts; these protoscolices and free daughter cysts are called hydatid sand. Each scolex normally invaginates to protect the hooklets (Fig. 17.3 and 17.4). Although not every cyst produces daughter cysts and/or protoscolices, this general tissue organization is called a unilocular cyst, in which the cyst contents are held within a single limiting cyst wall. On average, the cysts increase in size by about 1 cm per month; this growth rate was reported for a series of multiple intracranial hydatid cysts caused by *E. granulosus* (12).

When animals that serve as intermediate hosts are slaughtered, the viscera may not be disposed of properly and may be consumed by animals that serve as definitive hosts. The adult worms then develop in the intestine of the definitive host.

Based on differences in the host specificity of the larval stage, at least 10 different strains of *E. granulosus* have been identified (Table 17.2). The strains differ in their

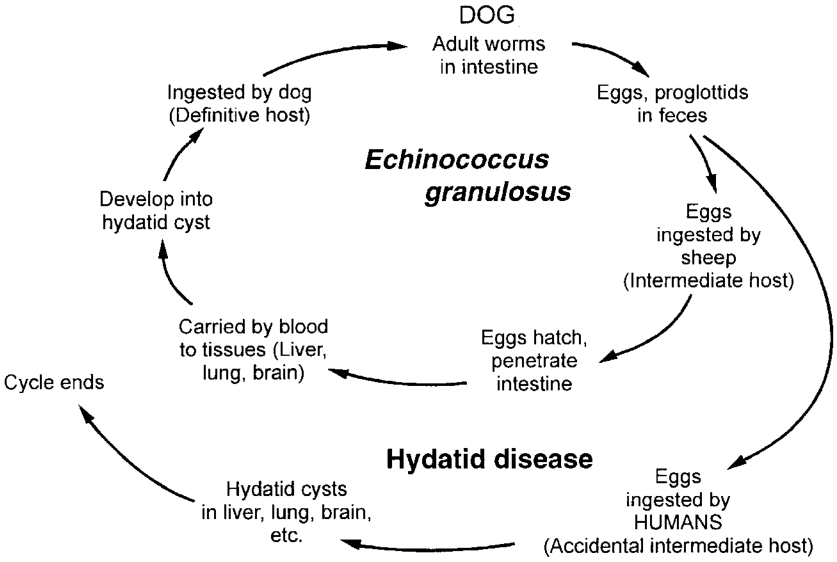
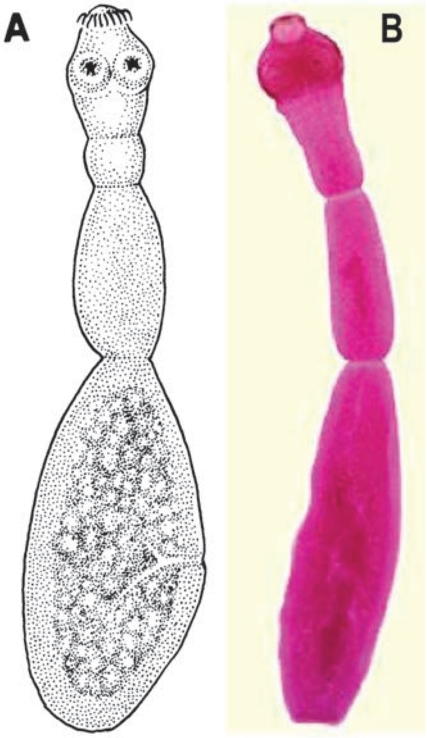

DOG
Adult worms in intestine
Eggs, proglottids in feces
Eggs ingested by sheep (Intermediate host)
Eggs hatch, penetrate intestine
Carried by blood to tissues (Liver, lung, brain)
Develop into hydatid cyst
Ingested by dog (Definitive host)
Echinococcus granulosus
Hydatid disease
Eggs ingested by HUMANS (Accidental intermediate host)
Hydatid cysts in liver, lung, brain, etc.
Cycle ends

Figure 17.1 Life cycle of *Echinococcus granulosus* (cystic echinococcosis).
doi:10.1128/9781555819002.ch17.f1

Figure 17.2 Adult worm of *Echinococcus granulosus*. (A) Diagram. (B) Whole mount (note that only three proglottids are present in the adult worm). (Whole mount courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch17.f2



intermediate host spectrum, geographic distribution, adult and larval stage morphologies, and protoscolex production. Also, at least seven of the strains are infective for humans. The northern form is found in the holoarctic zones of the tundra and boreal forest of North America and Eurasia. The larval stage occurs primarily in moose, elk, and reindeer, while the adult worm is found in wolves and sometimes in dogs and other wild canids. The European form of *E. granulosus* includes domestic animals, primarily sheep as the main intermediate host and dogs as the main definitive host. Although mixed infections with *E. granulosus* and *E. multilocularis* are rare, they have been reported from areas in China, a recognized area where echinococcosis is hyperendemic (13).

The risk of infection depends to a high degree on the association between humans and dogs, with the exception of the lion strain. Those at high risk include populations where dogs are used to herd sheep and are also intimate members of the family, often having unrestricted access to the house and family members. Cystic echinococcosis has been recorded in 21 of China's 31 provinces, autonomous regions, and municipalities (approximately 87% of the territory). This infection constitutes one of the major health problems in this part of the world. Hydatid disease caused by *E. granulosus* is a zoonosis of major public health concern throughout Latin America, particularly in the Andean and South Cone regions. Cystic echinococcosis is also widely found throughout the region comprising Arab North Africa and the Middle East. In areas of endemic infection around the world, the practice of giving raw viscera of slaughtered livestock to the dogs enhances transmission; in areas where this practice has been curtailed, prevalence figures have decreased.

Clinical Disease

Hydatid disease in humans is potentially dangerous; however, cyst size and organ location greatly influence the outcome. Clinical symptoms may appear after an incubation period of several months to years (Table 17.3).

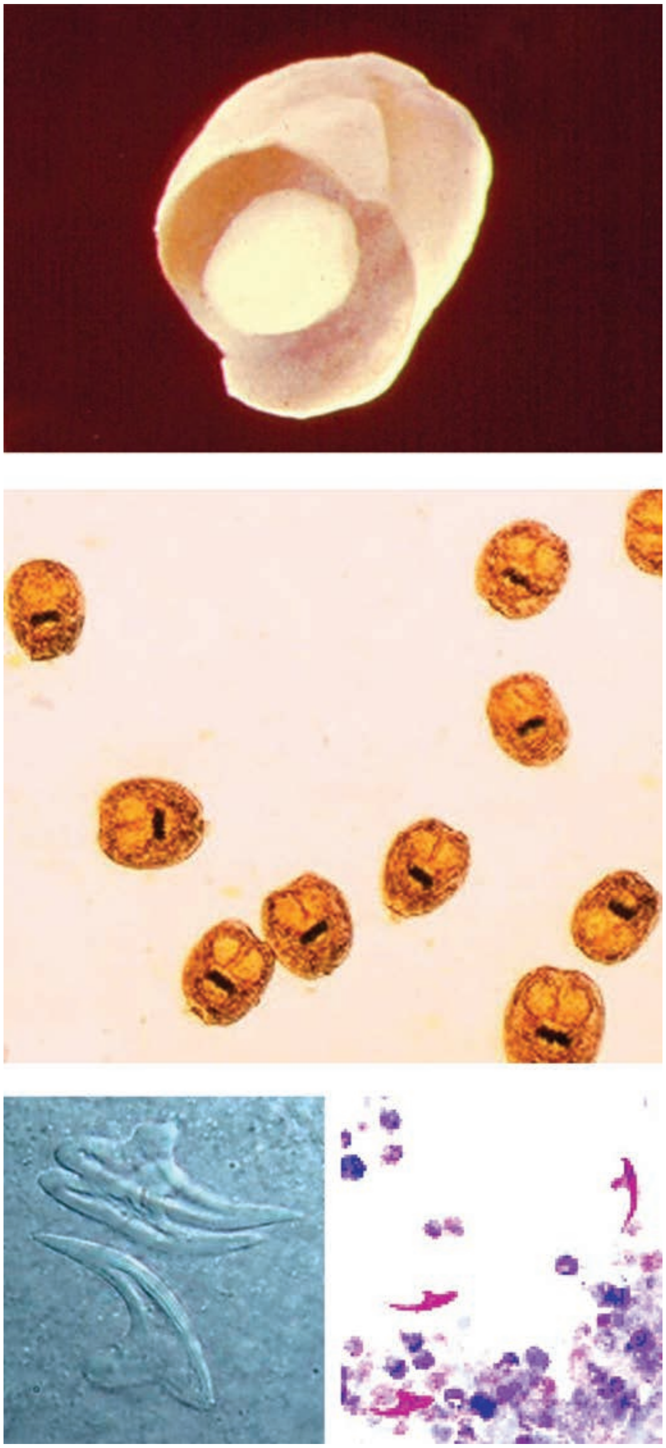


Figure 17.3 *Echinococcus granulosus*. (Top) Daughter cyst within the main cyst wall (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). (Middle) Hydatid sand (immature protoscolices). (Bottom) Individual hooklets: (left) unstained, interference microscopy (courtesy of the Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney); (right) hydatid hooklets revealed by Ryan modified blue trichrome stain (courtesy of Clavel A et al, *J Clin Microbiol* **37**:1561–1563, 1999); see also chapter 6. doi:10.1128/9781555819002.ch17.f3

Liver Cysts. Hepatic cysts are increasingly found as a mere coincidence on abdominal imaging techniques, such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). In approximately 90% of cases of echinococcosis, the primary presentation is a spherical, fluid-filled vesicle with an inner cellular layer and an outer laminated layer. The majority of hydatid cysts occur in the liver, causing symptoms that may include chronic abdominal discomfort, occasionally with a palpable or visible abdominal mass. Liver cysts tend to occur more frequently in the right lobe. Symptoms can include pain, hepatomegaly, cholestasis, biliary cirrhosis, portal hypertension, and ascites. If a cyst becomes infected with bacteria, it resembles an abscess. Severe symptoms can occur if the cyst ruptures spontaneously, from trauma, or during surgery. In addition to the spread of tissue from which additional hydatid cysts can grow, there may be serious allergic reactions, including rash, anaphylactic shock, or death. Cyst rupture can also lead to cholangitis and cholestasis (14). Morbidity and mortality rates associated with perforated hydatid cysts are higher than those associated with nonperforated cysts. Specific disease sites and complications can be seen in Table 17.3. Some unilocular cysts may remain undetected for many years until they become large enough to crowd other organs (Fig. 17.5).

Lung Cysts. The ratio of lung to liver involvement is higher in children than in adults (15). Cysts in the lungs are usually asymptomatic until they become large enough to cause chronic cough, shortness of breath, or chest pain (Fig. 17.6). Cyst rupture can lead to expectoration of hydatid fluid or membranes, followed by the development of infection and a lung abscess. If the rupture occurs into the lung, it may cause pneumothorax and empyema, allergic reactions, and even anaphylactic shock. When cysts rupture, either by spontaneous trauma or during surgery, protoscolices contained in the cyst fluid may disseminate, developing secondary cysts in the surrounding tissues.

Other Sites. The spleen and kidneys are the next most common intraabdominal organ sites, cysts occurring in 3

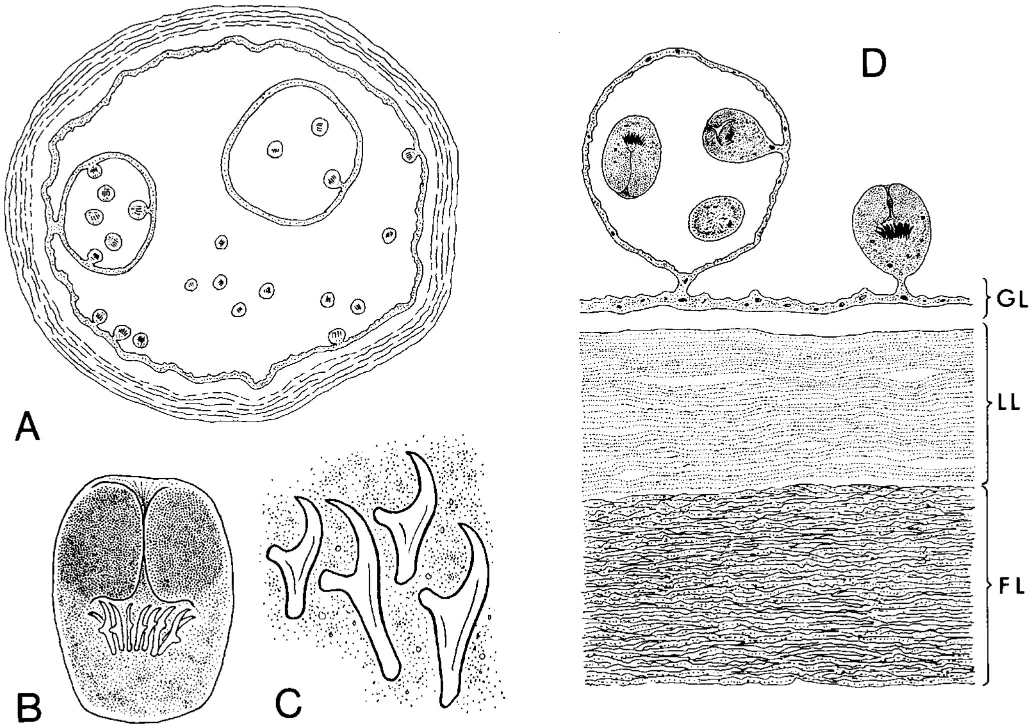


Figure 17.4 *Echinococcus granulosus* hydatid cyst. (A) Diagram of unilocular cyst (limiting membrane). (B) Diagram of single immature worm. (C) Diagram of hooklets. (D) Diagram of laminated layers, daughter cysts, and protoscolices. (Illustration by Sharon Belkin.) GL, germinal layer; LL, laminated layer; FL, fibrous layer. doi:10.1128/9781555819002.ch17.f4

to 5% of patients, and cardiac and cerebral cysts have been found in about 1 to 1.5% of patients (16). Even relatively small cysts can cause severe damage if they are located in a vital area or in bone (osseous cysts). Diagnostic procedures for the detection of liver or lung cysts should always be considered when cysts are detected in more unusual or rare body sites (Fig. 17.7).

Cyst Location Related to Patient Age. It is well known that hydatid cysts are most commonly found in the liver and lungs, but certain locations have been reported to be more prevalent in children and/or young adults. Lung, brain, spinal, and orbital hydatid cysts have been more commonly seen in younger patients. It is thought that age somehow alters the host-parasite relationship and thus affects the organ distribution of the cysts (17). Multi-organ involvement seems to be on the rise in children. Physicians need to be more aware of echinococcosis clinical features and to rule out other organ involvement once a cyst is detected in any body site.

Hydatid Disease and Cancer. The simultaneous occurrence of hydatid disease and cancer is relatively rare. In a patient who had liver disease as well as acute leukemia, therapy for his leukemia over a 3-month time frame had no effect on the size and contents of the liver hydatid cyst. The hydatid serologic titers remained unchanged, and the cyst classification remained the same (18). Hodgkin's lymphoma and cystic hydatid disease as a dual infection has been reported; however, no residual and/or new cyst formation was seen on follow-up after surgery and subsequent therapy for the lymphoma (19). Depending on the cyst site and symptoms, malignancy or echinococcosis may need to be differentiated.

Immune Reactions. During the life of the cyst, there may be small fluid leaks into the systemic circulation that sensitize the patient. Later, if the cyst should burst or if there is a large fluid leak, serious allergic sequelae, including anaphylactic reactions, may occur. Release of cyst tissue may lead to abscess formation, emboli, and/or the development of additional young cysts at secondary sites.

Although publications have described autoimmune phenomena in patients with hydatid cysts, apparently there is no association between hydatid infection and the level of autoantibodies to a broad range of self antigens such as antinuclear antibodies, tissue-specific autoantibodies, and rheumatoid factor. No significant differences have

TABLE 17.3 Examples of hydatid disease, complications, and/or outcomes[a]

Body site, description	Comments
Liver, asymptomatic disease	Despite a limited number of cases, most asymptomatic liver hydatid patients (75%) remain symptom free for >10 yr, regardless of cyst size or type. Such carriers are probably at low risk for developing complications.
Liver, surgical treatment	Most have cyst in right lobe, left lobe, both lobes; multiple hepatic cysts, coexisting cysts in other intra-abdominal sites. For management of infection, capitonnage (surgical closure), omentoplasty (use of portion of greater omentum used to cover or fill defect), cyst excision, segmentectomy, or cystoenterostomy (internal drainage of cysts into some portion of intestinal tract) are all superior to tube drainage.
Liver, right hepatic vein	Defect on wall of right hepatic vein, as well as three ruptures into the bile duct. Unusual presentation.
Liver, spontaneous rupture of cyst into biliary tract	Resolution of hydatid cyst by spontaneous rupture into the biliary tract; impaction of hydatid material into common bile duct can be relieved endoscopically; patient can also be treated with two courses of mebendazole.
Liver, intrabiliary rupture of cyst	Ideal treatment is traditional lavage of biliary tree followed by radical treatment of cyst and free drainage of bile ducts; hepatectomy and cystojejunostomy can be avoided.
Liver, cyst rupture directly into the left colon	Rupture of hydatid cyst into a hollow area such as the colon is extremely rare; partial drainage of the cyst was demonstrated by creation of an air-fluid level.
Intra-abdominal organs, spontaneous cyst rupture	Rare cases with severe spontaneous systemic anaphylactic reaction due to cyst rupture (without known precipitating factors such as trauma, accidental). Emphasizes importance of high index of suspicion for hydatid etiology for idiopathic anaphylactic reactions in areas of endemic infection.
Pancreas, rupture into main pancreatic duct	Recurrent pancreatitis due to rupture of a solitary hydatid cyst of the pancreas into the main pancreatic duct.
Lung, mean age of patients with giant hydatid cysts younger than patients with normal-size cysts	Increase in cyst diameter correlated with higher lung tissue elasticity and delay in diagnosis due to delayed symptoms.
Lung, secondary pleural disease (usually primary, not secondary)	Case presented 4 yr after treatment for a pyopneumothorax caused by rupture of pulmonary cyst near the pleural space; treatment with albendazole; surgery ruled out due to severe chronic obstructive pulmonary disease.
Unusual intrathoracic, extrapulmonary hydatid cysts	Sites included fissure, pleural cavity, chest wall, mediastinum, myocardium, diaphragm; total resection in 18/22 patients; no deaths after 1 yr.
Aorta, causing occlusion of aorta and both iliac arteries	Patient had previous surgery for a paraspinal hydatid cyst; presented with intermittent limping. CT and MRI showed multiple cysts in soft tissues of the back, retroperitoneum, and lumen of aorta and iliac arteries.
Heart, causing tamponade	Patient complained of severe dyspnea, thoracic pain, dizziness, short period of unconsciousness. Preliminary serology negative, low eosinophilia (5%); hydatid dismissed. Later, positive serology and eosinophilia of 59% found. Surgery and therapy with albendazole were used. With "cardiac tumor," negative serology and absence of eosinophilia do not rule out hydatid disease.
Heart, death caused by hydatid embolism	Rare case, limited to the heart; asymptomatic course until sudden death caused by arterial embolism. Note: no antibodies were detected by indirect hemagglutination.
Heart, cardiac involvement is usually by only 0.5 to 3% of hydatid cysts	Patient evaluated for intracardiac cyst; surgically excised; diagnosis confirmed by Giemsa staining of hooklets and scolices in cyst fluid. Patient was young (4-yr-old male).
Heart, in the septum interventriculare	Unusual location for hydatid cyst.
Heart (primary cyst), cerebral cysts (secondary cysts)	Rare case in 7-yr-old child; symptoms of raised intracranial pressure; underwent nine operations over 8 years because primary ruptured cyst in myocardium of left ventricle was detected; concurrent therapy with mebendazole (would not have been successful without removal of intracardiac hydatid cyst).
Heart, cyst located in septum and right ventricular cavity	Cyst located exclusively in the heart, first manifested as anaphylactic shock of unknown origin, required immediate surgical treatment due to severe hemodynamic compromise.
Orbital hydatid cyst	Emphasis on the superiority of MRI over CT for orbital cysts.
Central nervous system	Multiple hydatid cysts in cerebral subarachnoid space; based on clinical findings, latex test results, cerebrospinal fluid exam, and MRI findings.
Spine, progressive paraplegia	Rare hydatid disease manifestation. Disease misdiagnosed as tuberculous spondylitis. Patient presented with increasing weakness in lower limbs; treated for tuberculous spondylitis. Presented 1.5 yr later and underwent surgery and chemotherapy; paraparesis resolved. Early decompressive surgery with stabilization of the spine plus chemotherapy is treatment of choice.

(continued)

TABLE 17.3 Examples of hydatid disease, complications, and/or outcomes[a] *(continued)*

Body site, description	Comments
Spine, uncommon but significant	Review of 28 reports; only 14 patients had pulmonary or other organ involvement; most patients misdiagnosed as Pott's disease (tuberculous spondylitis); surgical intervention followed by chemotherapy; hydatid disease should be considered when radiologic findings suggest spinal infections or tumors.
Spine, rare case	Intraspinal extradural hydatid disease of the thoracic region with spinal cord compression; diagnosis on MRI findings; patient recovered completely after surgery.
Gluteus muscle	Unusual location for hydatid cyst.
Ovary	Asymptomatic right adnexal mass; vaginal ultrasound revealed cyst in posterior cul de sac adjacent to the right ovary, with internal septa resembling a maze or an onion slide structure. Color Doppler revealed simple echogenic pattern; in contrast, ovarian carcinomas have a complex internal structure.
Tongue	Unusual site (five reported cases in the literature); additional liver and cyst lesions found and treated with albendazole.
Seminal vesicle	Unusual site; include in differential diagnosis of a cystic lesion in pelvis especially in regions where hydatid disease is endemic.
Umbilical fistula	Rare case; spontaneous cutaneous fistulization of liver cyst very rare.
Bone	Rare (0.2–4%); requires high index of suspicion, prognosis often poor, especially in the spine; most patients do not recover neurologically; complication rate is high.

[a] See references 55–83.

been found between autoantibody levels in the sera from patients and from controls.

Echinococcus-specific IgE is present in most of the patients and is associated with the severity of disease. Specific histamine release by circulating basophils stimulated with *E. granulosus* antigens is present in all patients with cystic echinococcosis and alveolar echinococcosis. *Echinococcus* allergens include (i) AgB 12-kDa subunit, a protease inhibitor and a potent Th2 inducer; (ii) Ag5, a serine protease; (iii) EA 21, a specific cyclophilin, with homology to other types of cyclophilins; and (iv) Eg EF-1 beta/delta, an elongation factor with homology to *Strongyloides stercoralis* EF, which has the same IgE epitope. Clarification of the immunology of echinococcosis in humans has led to new therapy options, such as immunomodulation using alpha interferon. Also, a Th2-driven immunological response and an interleukin-10 (IL-10)-related tolerance state are common characteristics of atopic allergy and echinococcosis (20).

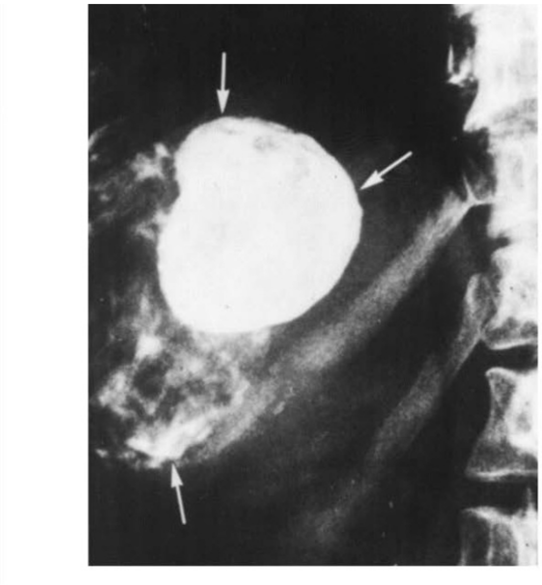
The hydatid cyst produces and exposes many immunomodulatory molecules to the host's immune system. During the past 30 years, studies on the immunobiology of *E. granulosus* have confirmed that parasite immunomodulating proteins actively interact with innate and adaptative human immune processes to reduce the impact of a host response. The major components of the immune response to hydatid cyst fluid in the host can be seen in Fig. 17.8 (21).

Diagnosis

Cystic echinococcosis diagnosis is primarily based on imaging techniques that use the World Health Organization international classification of ultrasound images (22). This classification is also useful in determining the type of cyst according to its activity (i.e., active: CE1, CE2; transitional: CE3a, CE3b; or inactive: CE4, CE5) and to monitor the clinical evolution of patients. In an area of endemicity, exposure to sheepdogs, an enlarged liver, the presence of a palpable mass in the right upper quadrant of the abdomen, expectoration of a salty-tasting fluid with hemoptysis, and a round lesion on chest radiographs all support the presumptive diagnosis of hydatid disease in either the liver or the lungs. Hydatid cysts in the liver may be confused with congenital liver cysts, amebic abscess, or hepatic tumors. Pulmonary symptoms may be confused with tuberculosis.

Hydatid cysts should be considered in patients with abdominal masses with no clearly defined diagnosis. Although eosinophilia is present in 20 to 25% of patients, it is merely suggestive. Many asymptomatic cysts are first discovered after radiologic studies. The cyst usually has a well-defined margin with occasional fluid level markings. These studies can also be helpful in diagnosing osseous involvement. Scans may also demonstrate a space-occupying lesion, particularly in the liver. If the cyst is large and located in the abdomen, a thrill is sometimes detected.

Laboratory and imaging data are usually sufficient to establish a diagnosis; however, they may also be inconclusive. Diagnostic confirmation is obtained using a combination of immunodiagnostic tests such as indirect hemagglutination (IHA), indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and immunoblotting. Some of the potential problems with these serological tests include (i) the lack of standardization of antigen preparations; (ii) performance using native or recombinant antigen, cyst fluid, or crude parasite preparations; (iii) potential low sensitivity; and (iv) potential cross-reactivity with other helminths.



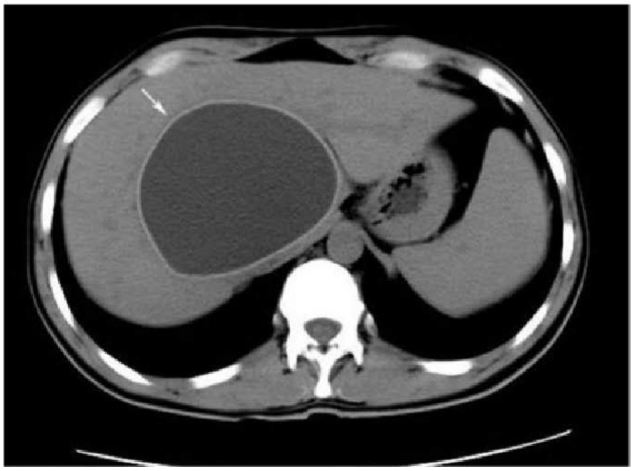


Figure 17.5 Hydatid cysts in liver. (Upper) This patient had three cysts (marked by arrows) in the right lobe. Two were completely calcified, and the large one in the background was partially calcified. (From Zaman V, *Atlas of Medical Parasitology*, Lea & Febiger, Philadelphia, PA, 1979.) (Lower) Hydatid cyst in the liver of an 11-year-old girl (courtesy of Yuksel M et al, *Korean J Radiol* 2007, www.openi.nlm.nih.gov). doi:10.1128/9781555819002.ch17.f5

One of the confirmatory tests consists of an *E. granulosus* hydatid fluid antigen ELISA (23). The sensitivity of the ELISA ranges between 50 and 98% and depends on the site, size, number, and stage of cysts. Another variable is the time between the beginning of therapy and the serum collection. All of these factors may explain the test sensitivity variability reported by different laboratories using the same antigen. The use of recombinant antigens for immunodiagnosis of cystic echinococcosis has yielded an overall sensitivity of 91.2% and a specificity of 93% and had the potential to indicate a cure in surgically treated patients (24). The 2B2t recombinant antigen performed better than the hydatid fluid with B2t antigens and the IHA commercial kit (Hydatidose Fumouze Kit; Fumouze Diagnostics, Levallois-Perret, France). Therefore, this new 2B2t-ELISA is a promising candidate test for the serodiagnosis of cystic echinococcosis in clinical settings (25).

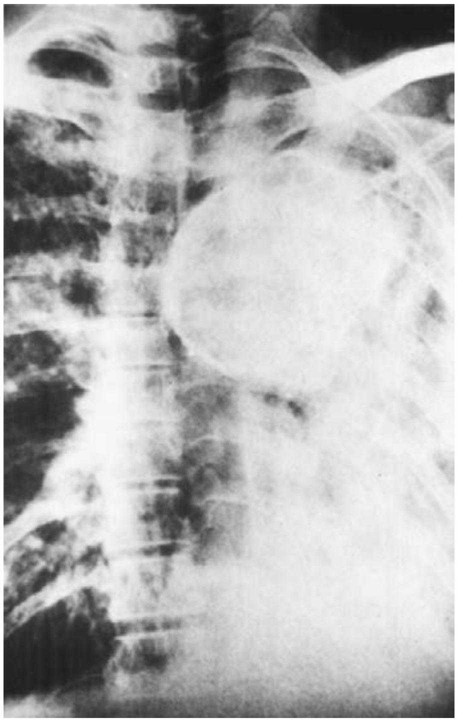


Figure 17.6 Hydatid cyst in the lung. This patient had a single large cyst in the left lung. There was collapse and consolidation in part of the left lung as a result of pressure on the bronchus. Partial pneumonectomy was required to remove the cyst. (From Zaman V, *Atlas of Medical Parasitology*, Lea & Febiger, Philadelphia, PA, 1979.) doi:10.1128/9781555819002.ch17.f6

Immunoblot is one of the most sensitive and specific methods and is normally used as a confirmatory test when the IHA and ELISA tests are inconclusive. A newer study has shown the ability of a human hydatid cyst fluid immunoblot (h-HCF-IB) test to recognize different band patterns from sera of patients with cysts in different locations; this provides new options for the clinical management of cystic echinococcosis and reduced follow-up time (26).

Serologic differentiation between cystic and alveolar echinococcosis, involving recombinant larval antigens, has also been used (27). Studies show good correlation between radiologic and serologic detection; however, serologic detection is more specific but less sensitive than imagery.

Once the cyst is discovered and surgical removal is selected as the approach, some of the cyst fluid can be aspirated and submitted for microscopic examination to detect

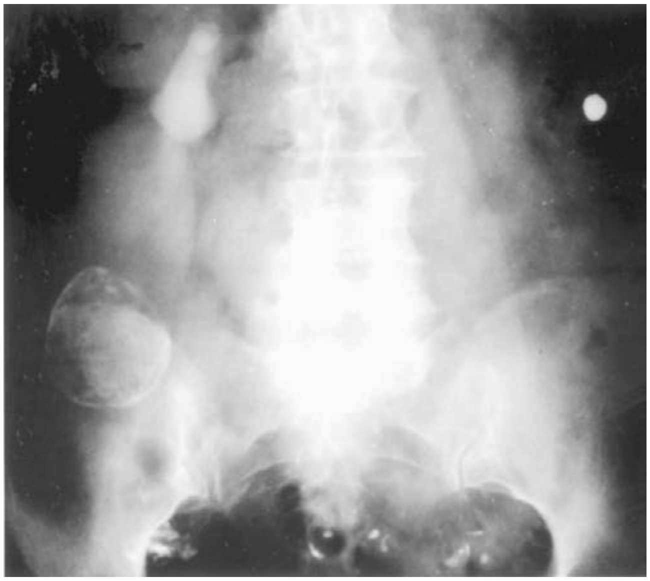


Figure 17.7 *Echinococcus granulosus* calcified liver cyst. The cyst is visible at the upper edge of the right pelvis; the dense pearlike structure at left is the gallbladder. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch17.f7

the presence of hydatid sand, thus confirming the diagnosis. This procedure is definitely not without risk because of possible fluid and/or tissue leakage or dissemination. Cyst aspiration is usually performed at the time of surgery.

Hydatid sand is not always present. Also, if the cyst is old, the daughter cysts and/or protoscolices may have disintegrated, so that only the hooklets are left. These may be difficult to find and identify if there is debris within the cyst (Fig. 17.3).

To detect hydatid sand, a drop of centrifuged fluid is placed on a slide and then another slide is placed on top of the drop. The two slides are then rubbed back and forth over the fluid. The grating of the calcified hooks on the glass may be felt and heard (hydatid sand sounds like glass grating on sand grains). Of course, if the individual protoscolices are intact, routine microscopic examination of the centrifuged fluid as a wet mount will confirm the diagnosis. If the cyst is sterile (no daughter cysts or protoscolices), the diagnosis could be confirmed histologically from the cyst wall. Refer to Algorithm 17.1. If the material is purulent, place a few milliliters of fluid into a tube and add concentrated hydrochloric acid (HCl). Either boil for 10 min or leave at room temperature for approximately 1 h until the material is quite fluid. Centrifuge and examine the material thoroughly for the refractile hooklets, which survive the HCl treatment intact. Along with the cellular and other mucoid material, the protoscolices are destroyed; however, the hooklets are liberated and can be seen and identified (personal communication from Graeme Paltridge).

KEY POINTS—LABORATORY DIAGNOSIS

Echinococcus granulosus (Cystic Disease)

1. Presumptive diagnosis may be based on history, radiographic studies, or scans.
2. Additional supportive data may be acquired from immunologic tests.
3. Microscopic examination of hydatid cyst fluid may reveal the hydatid sand (protoscolices) or, under certain circumstances, just the hooklets.
4. If the cyst fluid is thick or purulent, it may have to be subjected to a digestion procedure prior to examination; the hooklets survive this treatment and can then be seen and identified.
5. Light, fluorescence, and epifluorescence microscopy can be used to visualize the hooklets; some approaches require staining and some do not.

Treatment

A number of options are available for treatment of cystic echinococcosis, including surgery, puncture-aspiration-injection-reaspiration (PAIR), and chemotherapy. Surgery is generally considered the treatment of choice for a complete cure, although this approach is limited to unilocular cysts in operable body sites. In cases where multiple cysts are present in several different sites or in patients with a high surgical risk, PAIR and chemotherapy are considered appropriate options, either together or separately.

Surgery. Because there is always the risk of cyst fluid spillage during surgery, pre- and/or postoperative use of chemotherapy may be appropriate. Chemotherapy with albendazole or mebendazole offers the advantage of reducing the risk of disease recurrence and intraperitoneal seeding of infection that may develop via cyst rupture and spillage occurring spontaneously or during surgery or needle drainage. Surgery is normally reserved for patients with hydatid cysts refractory to PAIR due to advanced hydatid disease or secondary bacterial infection or for patients with cyst-biliary communication or obstruction.

Puncture-Aspiration-Injection-Reaspiration. PAIR is minimally invasive and includes the following steps: (i) under ultrasonographic guidance, percutaneous puncture of the cyst; (ii) aspiration of 10 to 15 ml of the cyst fluid; (iii) injection of a parasitocidal solution (95% ethanol in a volume one-third the amount of fluid aspirated); and (iv) reaspiration of the fluid after 5 min (2). Although hypertonic NaCl (15% [final concentration] in the cyst fluid) can be used instead of 95% ethanol, reaspiration of the fluid should not be performed until after 15 to 20 min. PAIR appears to have a higher incidence of cure, lower rates of complications, mortality, and disease recurrence, and fewer hospitalization days compared with surgery (28). Also, for patients with

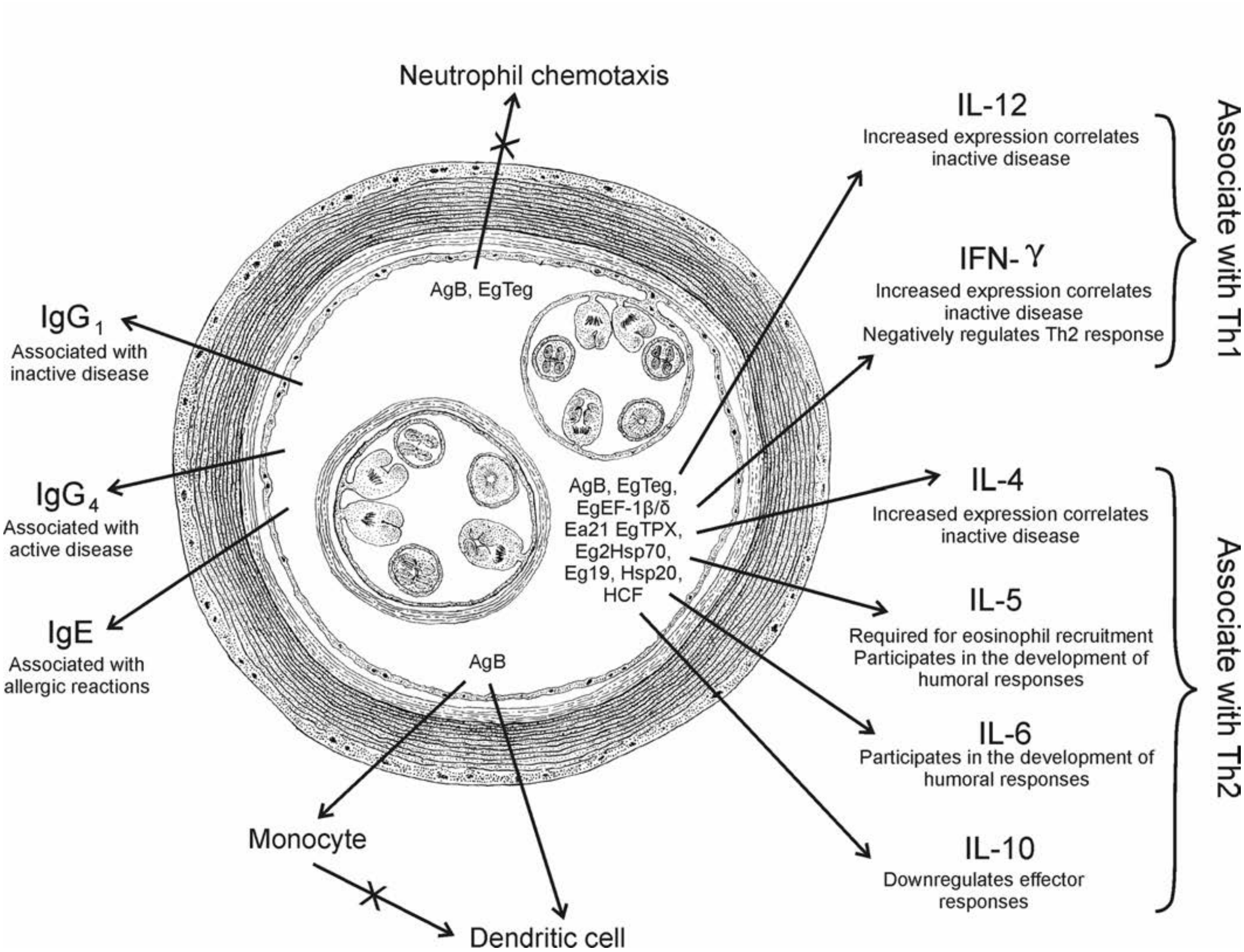

Neutrophil chemotaxis
AgB, EgTeg
IgG$_1$
Associated with inactive disease
IgG$_4$
Associated with active disease
IgE
Associated with allergic reactions
AgB, EgTeg, EgEF-1β/δ Ea21 EgTPX, Eg2Hsp70, Eg19, Hsp20, HCF
AgB
Monocyte
Dendritic cell
IL-12
Increased expression correlates inactive disease
IFN-γ
Increased expression correlates inactive disease
Negatively regulates Th2 response
Associate with Th1
IL-4
Increased expression correlates inactive disease
IL-5
Required for eosinophil recruitment
Participates in the development of humoral responses
IL-6
Participates in the development of humoral responses
IL-10
Downregulates effector responses
Associate with Th2

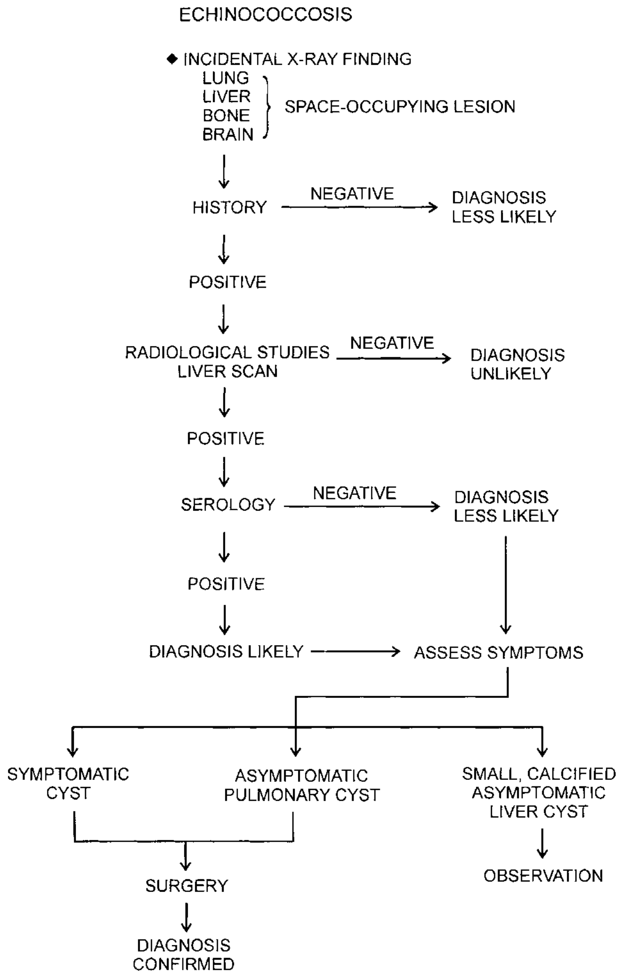
Figure 17.8 *Echinococcus granulosus*. Major components of the immune response to hydatid cyst fluid in the host: *Echinococcus granulosus*-derived immune modulators and the main cytokines that regulate this response. Parasite-derived molecules as AgB, EgTeg, and EgEF-1β/δ could elicit a predominant Th2 activation, whereas EgTPx and other HCF components can elicit a concomitant Th1/Th2 cell activation. (Courtesy of Siracusano A et al, *Clin Dev Immunol* **2012:**639362, 2012.) doi:10.1128/9781555819002.ch17.f8

hepatic echinococcosis who fail to respond to chemotherapy alone, PAIR is a safe and effective procedure. It is recommended for treatment of hepatic cysts of ≥5 cm in diameter, for cysts with daughter cysts, for cysts with detached membranes, and for multiple cysts accessible to puncture. Contraindications are for cysts communicating with the biliary tree, hepatic cysts with high surgical risk or in inaccessible locations, cysts free in the abdominal cavity, and cysts in the lungs, heart, brain, or spine (2).

To prevent chemical cholangitis, it is critical that PAIR not be used to treat cysts with biliary communication. Cyst aspirates must be checked for traces of bilirubin; if it is found in the cyst fluid, the PAIR protocol must be stopped immediately.

Percutaneous Thermal Ablation. Percutaneous thermal ablation of the cyst germinal layer using a radiofrequency ablation device has proven to be a newer option that may find greater use in the future (2). One advantage of this approach is that it avoids injection of parasitocidal fluids into the hydatid cyst.

Chemotherapy. During the last few years, experience with the benzimidazole derivatives mebendazole and albendazole suggests that under certain circumstances, chemotherapy may replace surgery. This approach is recommended for patients with inoperable cystic disease and those with multiple cysts in several body sites; however, chemotherapy for bone disease is less effective. Although relapses after


ECHINOCOCCOSIS
◆ INCIDENTAL X-RAY FINDING
LUNG
LIVER
BONE
BRAIN
SPACE-OCCUPYING LESION
HISTORY
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RADIOLOGICAL STUDIES LIVER SCAN
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
SEROLOGY
NEGATIVE
DIAGNOSIS LESS LIKELY
POSITIVE
DIAGNOSIS LIKELY
ASSESS SYMPTOMS
SYMPTOMATIC CYST
ASYMPTOMATIC PULMONARY CYST
SMALL, CALCIFIED ASYMPTOMATIC LIVER CYST
SURGERY
OBSERVATION
DIAGNOSIS CONFIRMED

Algorithm 17.1 Echinococcosis.
doi:10.1128/9781555819002.ch17.Alg17.1

chemotherapy have been reported, patients are usually sensitive to retreatment (2, 28). In general, side effects are mild.

In a series of 721 cases of bone echinococcosis, most infections involved a single bone (602/721; 83%) and often the spine (321 cases; 45%). In eight cases (8/702; 1%), a secondary bacterial surgical site infection was reported. Surgery was performed in 702 cases (97%), with single intervention in 687 episodes (95%). Complete excision of the lesion was possible in only 117 episodes (16%). Albendazole was by far the most frequently used agent in monotherapy with various dosages, while mebendazole in monotherapy was less frequent (32 cases). The median duration of anthelmintic therapy was 6 months (range 0.7 to 144 months). There were 124 recurrences (17%) after a median delay of 2 years (range 0.4 to 17 years) (29).

Immunologic markers indicating the effectiveness of pharmacologic treatments with albendazole and mebendazole in human hydatid disease have been reviewed. These studies show that IgE may be a useful marker of therapeutic success in patients with pretreatment specific IgE antibodies, since the levels of these antibodies decrease rapidly in those who respond fully to therapy. IgG subclass responses and differential immunoglobulin in subclass binding pattern to hydatid antigens may also be useful (30).

Epidemiology and Prevention

The percentage of infected hosts varies throughout the world, but human infection is still much less common than infection of any of the reservoir hosts. Areas of endemicity of growing concern are the sheep- and cattle-raising areas of Argentina, Uruguay, southern Brazil, and Chile (31). There are two life cycles of *E. granulosus*, the first of which can be domestic, involving the domestic dog as the definitive host and various species of domestic ungulates as intermediate hosts. The second type of life cycle is sylvatic, in which wild carnivores serve as definitive hosts and wild ungulates serve as intermediate hosts. The dog-sheep strain is the most common and important throughout the world. There are often overlapping domestic and sylvatic cycles, all of which present special situations and challenges related to disease control.

Most human hydatid cysts are probably acquired in childhood and may continue to grow undetected for many years. Several preventive measures are considered mandatory if the incidence is to decrease. First, all infected viscera from slaughtered animals (intermediate hosts containing hydatid cysts) must be disposed of so that dogs do not have access to this material. Also, personal hygiene must be emphasized to prevent accidental ingestion of the infective eggs from soil contaminated with dog feces, particularly since the eggs are very resistant to disinfectants. In many areas, extensive control measures have also been taken to reduce hydatid disease. Certainly, educational programs are also very important.

ELISAs for *Echinococcus* coproantigen have been developed to detect infection in animals (2, 32). Antigen could be detected for 6 months in fecal samples stored in 5% formalin. Improvements in sensitivity in patients with low worm burdens and modifications for field application may prove to be very helpful in future epidemiologic studies.

Echinococcus multilocularis (Alveolar Disease, Hydatid Disease)

For many years, pathologists recognized the difference between the unilocular type of human hydatid cyst and the alveolar form. Some workers thought that the alveolar form was a variant species, while others thought that it might arise from an early unilocular cyst. Investigations in Alaska and Germany served to define the characteristics of alveolar hydatid disease caused by *E. multilocularis*

(Table 17.1). Alveolar hydatid disease is the most lethal of helminthic diseases, with radical surgery still being the only curative therapy. However, resection has been possible in only 25 to 57% of patients. Most cases are diagnosed in rural residents, suggesting direct or indirect contact with fecal material from infected foxes or dogs. The spread of infections and the increasing numbers of documented cases in the former USSR, China, Japan, and Alaska are causing concern among public health personnel in these regions. There is some variation among isolates from North America and Eurasia; however, there appear to be no actual genetic strain differences (33).

Life Cycle and Morphology

The life cycle is essentially identical to that of *E. granulosus*, with the following exceptions: the definitive hosts in the sylvatic cycle tend to be dogs, cats, wolves, and foxes while the intermediate hosts have been identified as voles, field mice, house mice, ground squirrels, and shrews (Fig. 17.9). Laboratory infections have also been maintained in deer mice and gerbils.

The adult worms are smaller than those of *E. granulosus* (1.2 to 3.7 mm versus 3.0 to 6.0 mm long). Also, the eggs are *Taenia*-like and very resistant to cold.

The cyst itself is composed of many irregular cavities with little or no fluid, rare or no free protoscolices, and often central necrosis and cavitation of the lesion (Fig. 17.10). The human lesion is an example of persistent parasitic growth at a low level rather than active growth or malignancy.

Clinical Disease

Although the alveolar form of hydatid disease has been found in other tissues (eye, bone, lung, peritoneum, adrenals, brain, ovary, lymph nodes, prostate, gallbladder, pancreas, spleen), the liver is the most common site. The disease may resemble a slowly growing carcinoma, may cause symptoms of intrahepatic portal hypertension, and may spread to other sites via metastatic dissemination. In humans there are several stages of the infection; they have been described as initial, progressive, advanced, stable, and abortive course (2) (Table 17.4).

Proliferation of the cyst is most active at the advancing margin, while the interior of the cyst structure may deteriorate and appear to be a necrotic abscess filled with pus. The lesions tend to spread by direct extension into the surrounding tissues and can also be transported to other body sites via the lymphatic or hematogenous routes. In about 2% of cases, pieces of the germinal membrane can metastasize to other body sites, including the brain, lungs, and mediastinum. Since the human is not a normal host, the typical brood capsules, protoscolices, and calcareous corpuscles may not develop (Fig. 17.10). In the human, the laminated membrane may surround vesicles that are void of protoscolices and other internal structures. The lesions caused by *E. multilocularis* may appear as firm, solid, cancerlike masses, and the outer layer has a poorly defined margin that continues to grow and spread throughout the tissue.

In well-established disease, there may be hepatomegaly, splenomegaly, and finally jaundice and ascites. Patient symptoms include right upper quadrant pain and the presence of a palpable hepatic mass. There may also be shortness of breath, as well as central nervous system symptoms from brain metastases. Because the growth of the alveolar cyst is very slow, some patients may be infected for 20 or 30 years before exhibiting any symptoms. In patients who remain untreated, the mortality rate can exceed 90%.

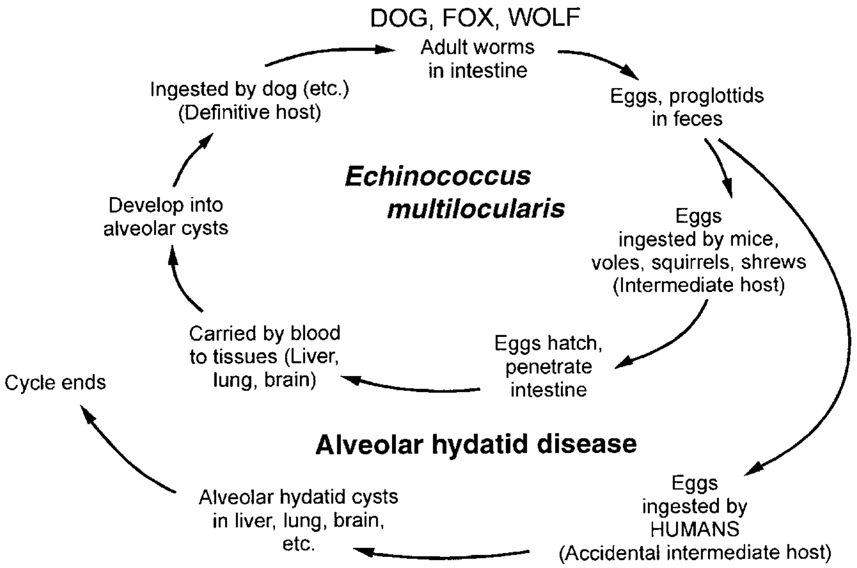
DOG, FOX, WOLF
Adult worms in intestine
Eggs, proglottids in feces
Eggs ingested by mice, voles, squirrels, shrews (Intermediate host)
Eggs hatch, penetrate intestine
Carried by blood to tissues (Liver, lung, brain)
Develop into alveolar cysts
Ingested by dog (etc.) (Definitive host)
Echinococcus multilocularis
Eggs ingested by HUMANS (Accidental intermediate host)
Alveolar hydatid cysts in liver, lung, brain, etc.
Cycle ends
Alveolar hydatid disease

Figure 17.9 Life cycle of *Echinococcus multilocularis* (alveolar hydatid disease). doi:10.1128/9781555819002.ch17.f9

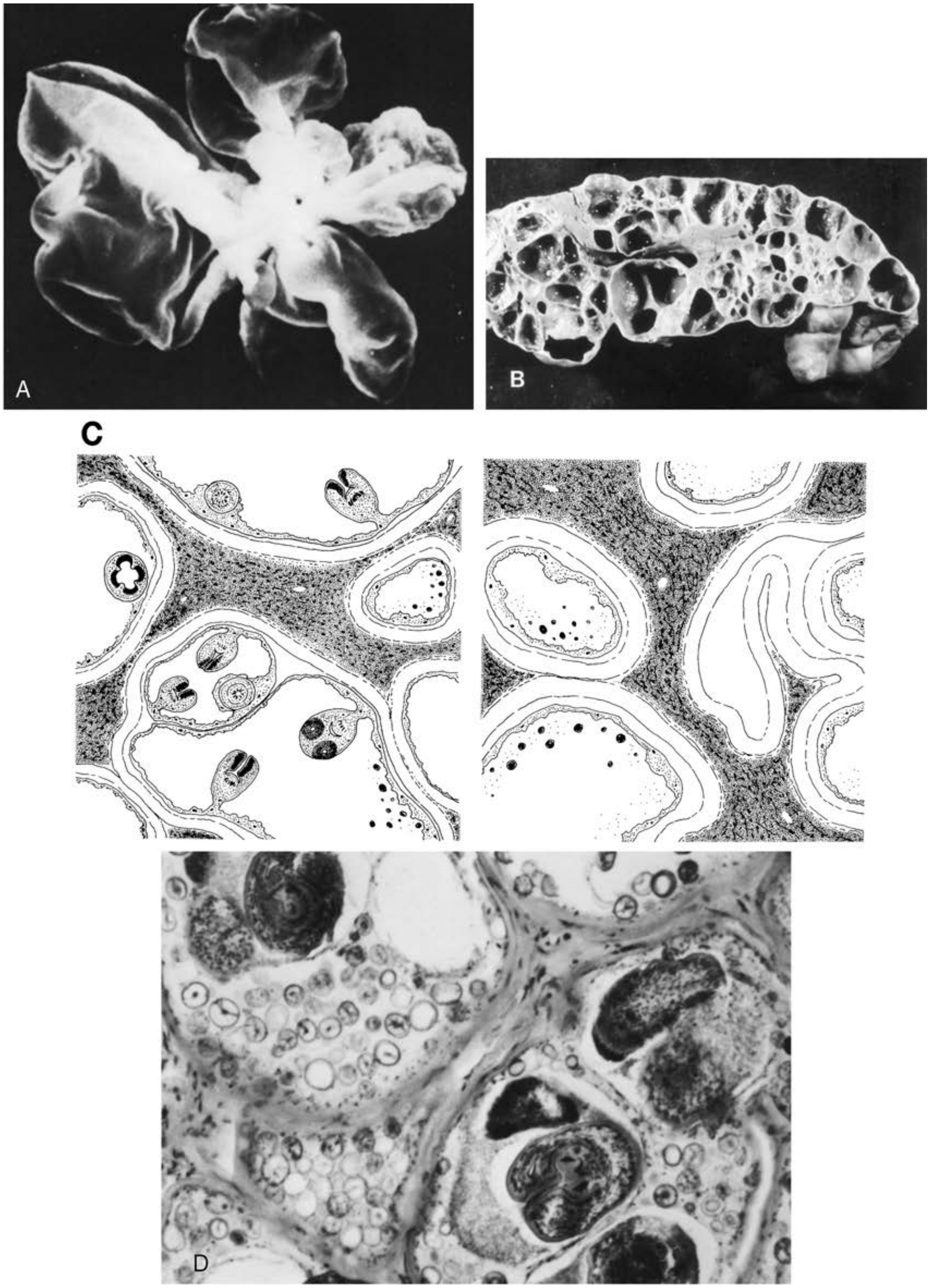

Figure 17.10 *Echinococcus multilocularis*. (A) Entire cyst. (B) Multicystic nature of the parasite in an experimental infection in a muskrat. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of R. L. Rausche.) (C) Diagram of a histologic section. The left side represents the normal animal intermediate host in which protoscolices are found; the right side demonstrates the cyst structure within the human accidental intermediate host in which the protoscolices may not develop. (Illustration by Sharon Belkin.) (D) Histologic section.

doi:10.1128/9781555819002.ch17.f10

TABLE 17.4 Infection phases: alveolar echinococcosis

Disease phase	Symptoms	Comments
Initial	Asymptomatic	Spontaneous cure or become progressive: incubation period 5–15 yr.
Progressive	Abdominal pain, jaundice, hepatomegaly, fever, anemia, weight loss, pleural pain	Symptoms occur when metacestode infiltrates larger parts of liver or impacts organ functions.
Advanced	Severe hepatic dysfunction, portal hypertension	Disease duration variable between weeks and years; mortality in both treated and untreated patients can be high.
Stable	Patients on long-term chemotherapy	Parasite growth inhibited.
Abortive	Seen in asymptomatic patients	Parasite dies and the cyst is calcified.

Studies directed at the metacestode developmental stage of *E. multilocularis* have confirmed that the 14-3-3 protein is found in the germinal layer and is expressed about 10-fold in this stage compared with the adult worm. These results, taken together with current knowledge of the 14-3-3 protein family, suggest that this parasite molecule may contribute to the promotion of the progressive, potentially unlimited growth behavior of the organism within the host tissue (34). Studies also suggest that viable *E. multilocularis* vesicles induce significant cellular production of cytokines and chemokines. In patients with viable alveolar hydatid disease, these immune mediators may enhance antibody-dependent cellular effector mechanisms against proliferating metacestodes (35).

Both humoral and cell-mediated immune responses occur. In the mouse model, there is an initial Th1-dominant cytokine response, including IL-2 and gamma-interferon (IFN-γ). This response is associated with diminished parasite growth. Rapid organism growth was associated with secretion of Th1- and Th2-type cytokines including IL-5 and IL-10. In patients with progressive disease, IL-10 was more pronounced and IL-10 levels in serum were higher than in patients with the abortive form (36).

Diagnosis

Imaging Techniques. Ultrasonography, in association with CT, is recommended; MRI can also be helpful. These imaging options can detect the majority of lesions, including those that have calcified. In a recent study, contrast-enhanced power Doppler ultrasonography was compared with CT for the diagnosis of alveolar echinococcosis. The three-phase helical CT was able to visualize the vascularization associated with *E. multilocularis* lesions in the liver, while neither unenhanced nor contrast-enhanced ultrasonography in power mode was suitable for this purpose (37).

Imaging techniques of the liver commonly reveal ill-defined lesions of the parenchyma, and contrast CT and MRI clearly demonstrate the infiltrative structure and extension of the parasitic tumor to adjacent structures. In histologic sections of the human liver, the larval parasite usually exhibits an alveolar (spongy) structure composed of numerous irregular vesicles with diameters between less than 1 and up to 20 mm (Fig. 17.11). These lesions behave like a slow-growing liver cancer, without sharp boundaries between the parasitic tissue and the liver parenchyma.

Immunodiagnostic Tests. The diagnosis of alveolar hydatid disease may be difficult unless there is a presumptive diagnosis based on history. Often, the correct diagnosis is missed even at autopsy. Serologic procedures used for unilocular

Figure 17.11 *Echinococcus multilocularis* (alveolar hydatid cyst). (Upper) H&E. Note the numerous irregular vesicles (quite different from a single limiting capsule cyst—cystic hydatid with *E. granulosus*) (courtesy of the CDC Public Health Image Library). (Lower) Periodic acid-Schiff staining of the laminated layer (courtesy of Bernadette Kantelip, http://emedicine.medscape.com/article/214349-workup#showall).
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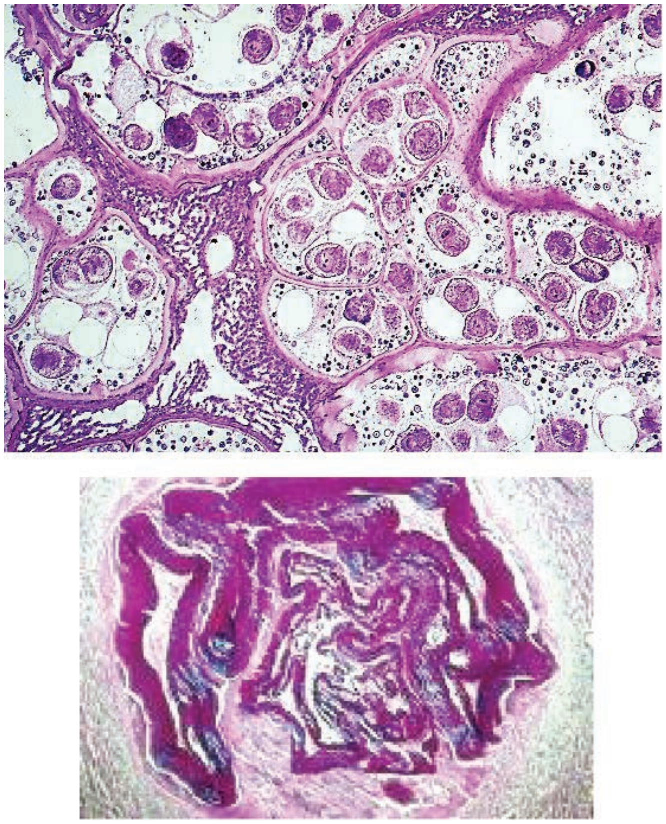


hydatid disease may be helpful (2, 38). The metacestodes of *E. multilocularis* contain glycolipids which are recognized by serum antibodies from patients with alveolar hydatid disease. The ELISA procedure results were confirmed by immunostaining on thin-layer chromatography (39). This approach may also provide another procedure for the diagnosis of alveolar hydatid disease. The use of monoclonal antibodies in a sandwich ELISA has also proven valuable in the diagnosis of alveolar hydatid disease, particularly when one is distinguishing this infection from those caused by other helminths. In general, one of the difficulties with any complex parasite involves the cross-reactivity seen when crude antigen extracts or antigen fractions from different isolates are used for immunodiagnosis. This approach results in a lack of test standardization and inability to interpret and compare test results. Because serodiagnosis fails in 5 to 10% of cases and radiologic techniques are difficult to interpret in some cases, PCR has been developed for the detection of *Echinococcus*-specific mRNA from fine-needle aspiration biopsy specimens (40). A PCR protocol for the detection and differentiation of *E. granulosus* and *E. multilocularis* in formalin-fixed, paraffin-embedded tissues has also been developed (41).

Fine-Needle Aspiration. Fine-needle aspiration biopsy findings for hepatic *E. multilocularis* have been reviewed; there appear to be some basic diagnostic criteria for this approach (42). Cytologic smears were stained with May-Grunwald-Giemsa and periodic acid-Schiff (PAS) stains. Tissue sections were stained with hematoxylin and eosin and with PAS. In tissue sections, homogeneous, thin cystic structures of various dimensions were strongly PAS positive. Mucoid material within the cystic structures was also PAS positive. Wide coagulative necrosis was seen in all cases, and there were foreign-body-type giant cells at the periphery of the lesion in some cases. In all of the cytologic smears, there were necrotic ground, PAS-positive hyaline cuticular structures, and mucoid globules; in some smears, there were also foreign-body-type giant cells.

In another case where the patient presented with a multicystic mass in the pancreas, fine-needle aspiration biopsy revealed some protoscolices, free hooklets, and fragments of the laminated layer, findings which are pathognomonic for echinococcosis. In this case, the patient had pancreatic alveolar hydatid disease (43). Serologic testing using an ELISA with Em2(plus) antigen showed high antibody reactivity with the patient serum, a finding indicative of an *E. multilocularis* infection. Diagnosis was confirmed by molecular analysis of the cytologic material using PCR and a direct fluorescent-antibody assay.

KEY POINTS—LABORATORY DIAGNOSIS

Echinococcus multilocularis **(Alveolar Hydatid Disease)**

1. Serologic results may be helpful (the same procedures as used for unilocular disease).
2. Histologic identification may be unsuccessful at both the biopsy and autopsy phases of the diagnosis as a result of nonrecognition of the etiologic agent or insufficient tissue. PAS stains can be helpful.
3. Imaging techniques can be very helpful, particularly when coupled with immunodiagnostic tests for confirmation.

Treatment

Surgery. Total surgical removal of an alveolar hydatid cyst is almost impossible, since the cyst morphology does not have a limiting capsule. However, in cases diagnosed in the early stages, radical surgery may result in a cure. In more advanced cases of alveolar disease, the effectiveness of radical surgery drops dramatically. Liver transplantation is generally not recommended, and there remains a risk of metastasis after surgery.

Chemotherapy. In addition to surgery, the same drugs that have been used for *E. granulosus* have been tried. Mebendazole and albendazole are the drugs of choice for treating alveolar echinococcosis. However, if one considers cost, albendazole reduces costs by about 40% and is easier for patients to take (44). In some situations, the data indicate that mebendazole therapy is parasitostatic rather than parasitocidal; however, even with recurrence, the patients responded favorably to reintroduction of chemotherapy. Because IFN-γ and IFN-α levels are decreased in patients with echinococcosis, the addition of IFN-γ to standard therapy with mebendazole has been reported to halt disease progression (45). The overall prognosis for this infection is usually very grave. However, spontaneous death of *E. multilocularis* cysts in humans has been reported.

Assessment of chemotherapy can be difficult and is usually based on the following criteria: (i) long-term patient survival rates, (ii) lack of disease progression, (iii) improved clinical status, (iv) slowed or stopped parasite proliferation, and (v) absence of disease recurrence. Currently, the approach has become very similar to the management of a patient with cancer. It includes a multidisciplinary approach with a number of options entering the discussion (type, size, number, anatomical location, potential changes in parasite lesions, patient characteristics).

Treatment with benzimidazoles for several years, or perhaps life, is mandatory in all inoperable alveolar hydatid patients and following incomplete surgical resection of the lesions. Therapy should be given for at least 2 years after radical surgery, and patients should be monitored for at least 10 years for possible recurrence (46). Although presurgical therapy is usually not indicated, some patients have been treated for months to years pending liver transplantation. Although praziquantel as an additional drug has been mentioned in the past, it really has no role in the treatment of alveolar echinococcosis.

Epidemiology and Prevention

A number of variables play roles in the epidemiology of *E. multilocularis* infection. Also, there are serious questions regarding the emergence and geographic spread of this infection. It is found in at least 30 countries, including Russia, Kazakhstan, China, parts of Europe, and North America. Although it appears that areas of endemic infection are increasing in size, it is difficult to tell whether the data reflect actual changes in the parasite range or merely clarification of range extensions that were already present but unrecognized.

Handling of infective definitive hosts or ingestion of contaminated food or water probably initiates the infection, while accidental ingestion of soil, berries, or other vegetables contaminated with eggs may be less important in transmission. However, it is important that the dangers of playing in the woods, picking berries, etc., in areas of endemicity that are frequented by wolves, foxes, and dogs be recognized, although routes of infection are difficult to prove due to the long incubation periods for the infection and lack of detailed information. Educational programs should be initiated early with children, since most infections are presumed to be acquired in childhood. There are probably additional factors that influence human infections but remain unknown.

The use of coproantigen testing by a sandwich ELISA method to detect infection in foxes is more sensitive than routine egg detection assays and has been recommended for diagnosis in the definitive host of *E. multilocularis*. Coprodiagnosis by PCR is an excellent alternative to necropsy, since the sensitivity of necropsy is no higher than 76%. The PCR system is an alternative, sensitive method for the routine diagnosis of *E. multilocularis* in carnivores.

Chemotherapy with praziquantel has the potential to reduce the prevalence of *E. multilocularis* in wild foxes. However, the question of its long-term efficacy and other unresolved problems such as cost need to be addressed by additional studies before routine application of this baiting method can be recommended.

Considering the high reproduction of *E. multilocularis* in domestic dogs which live in close contact to humans, a monthly deworming scheme for domestic dogs with access to rodents is likely to be very important. However, this is true if only low prevalences in domestic dogs are recorded, as high densities of these pets can easily outweigh low infection rates.

Echinococcus vogeli (Polycystic Hydatid Disease) and *Echinococcus oligarthrus* (Unicystic Hydatid Disease): Neotropical Echinococcosis

Approximately 200 cases of polycystic echinococcosis have been documented from 12 countries in South America (Table 17.1). Following early proliferation of *E. vogeli* in the human host, typically in the liver, the metacestode usually spreads in the peritoneal and pleural cavities, and numerous organs may be invaded. The clinical findings of polycystic disease in 81 patients with sufficient information were reviewed (47). Type I disease consists of polycysts in the liver and abdominal cavity (37% of the patients had this characteristic); type II is similar to type I but also includes hepatic insufficiency (26%); type III consists of cysts in liver and chest (14%); type IV consists of cysts only in the mesenteries (16%); and type V consists of cysts calcified in liver and lung (4%). The percentage of patients with polycysts in the liver was 81%, and the percentage of patients with polycysts in the chest was 14% (47).

A reevaluation of the characteristics of the metacestode of *E. oligarthrus* indicated that it is unicystic, rather than polycystic. Only three human cases are known (two with infection in the orbit and one with infection in the heart). The metacestode of *E. oligarthrus*, in contrast with that of *E. vogeli*, consists of a spherical, fluid-filled vesicle that enlarges concentrically and is not known to undergo exogenous proliferation. Although the metacestode of *E. oligarthrus* is unicystic, it can be single or multiple; however, when multiple, each cyst is separate and independent, and it does not proliferate in the human host but its structure in humans is apparently identical with that in the natural intermediate host.

Life Cycle and Morphology

E. vogeli and *E. oligarthrus* are the only indigenous species known in the neotropical area of the New World (47). The definitive hosts of *E. vogeli* include the bush dog, while the intermediate hosts harboring the larval forms (hydatid cysts) include the paca, agouti, spiny rats, and opossums. The *E. oligarthrus* life cycle involves wild felids such as pumas, jaguars, and wild cats as definitive hosts, while the intermediate hosts are the same as for *E. vogeli*. Further studies to determine which other wild animals might be involved in the life cycle are under way. The domestic dog is the most significant source of human infections with cystic, alveolar, and polycystic forms of echinococcosis.

Clinical Disease

Echinococcus vogeli. In the human host, proliferation of *E. vogeli* vesicles leads initially to extensive involvement of the liver. After an undefined interval, the proliferating vesicles penetrate Glisson's capsule and invasion of organs in the peritoneal and pleural cavities takes place. Information from an outbreak of polycystic echinococcosis in large primates at the Los Angeles Zoo suggests that advanced disease in humans may develop over a period of about 10 years.

E. vogeli is the etiologic agent of polycystic disease in humans in areas such as Brazil, Colombia, Ecuador, Panama, and Venezuela. Nine patients from Brazil were found to have polycystic hydatid disease, and the diagnosis was based on the shape and dimensions of the rostellar hooks. The liver was the most common site, followed by the lungs, mesentery, spleen, and pancreas. The main symptoms included abdominal pain, hepatomegaly, jaundice, weight loss, anemia, fever, hemoptysis, palpable abdominal masses, and signs of portal hypertension. The most common clinical presentation involved the abdomen, with hard, round masses in or connected to the liver. In 25% of cases, there were also signs of portal hypertension; all of these patients died, either from the disease or from surgical complications. Asymptomatic individuals accounted for 10% of the cases. Hepatic calcifications were seen in four patients. Patients may have calcified, round structures in the liver, suggestive of calcified polycystic hydatids. This disease is chronic and can last for over 20 years; in advanced cases the mortality is high.

Echinococcus oligarthrus. Similar to infections with *E. granulosus*, the oncospheres of *E. oligarthrus* may pass through the liver and be transported by the systemic circulation to various loci in natural, as well as atypical, intermediate hosts. At the present, in humans, only three cases of unicystic echinococcosis have been recognized; thus, the potential range of loci in the human body is not known. In humans, the metacestode of *E. oligarthrus* has been described as a more or less spherical structure that enlarges concentrically, and pathogenesis appears to result from pressure effects caused by its expansion. Two of the three known cases involved retro-ocular location of single vesicles, with production of significant exophthalmos (48, 49). In the third case, the autopsy of a patient who died of tetanus at 70 years of age revealed two metacestodes, each about 1.5 cm in diameter, in the left wall of the cardiac ventricle (50). In those patients, development of the metacestode was evidently normal, and the rostellar hooks of protoscolices provided the means of identification of the organism as *E. oligarthrus*. This patient also had chagasic myocarditis (*Trypanosoma cruzi*).

In a case of human hydatid disease caused by *E. oligarthrus*, the patient presented with proptosis of the left eye and persistent headache; a CT scan revealed a single orbital, retro-ocular cyst approximately the same size as the eye and thought to be a tumor. During surgery, fluid and internal tissues were taken from the cyst, which was also later removed. A few protoscolices and hooklets were found, and *E. oligarthrus* was identified (Fig. 17.12 and 17.13). The second case was from a 6-year-old child with irreducible exophthalmos; only light reception functioned.

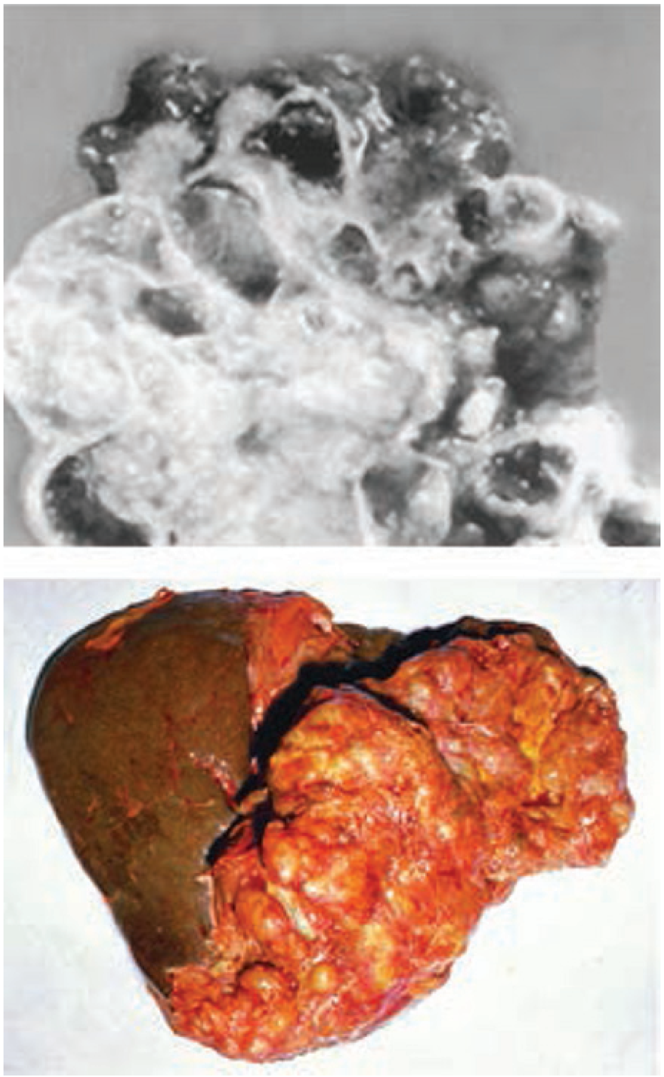
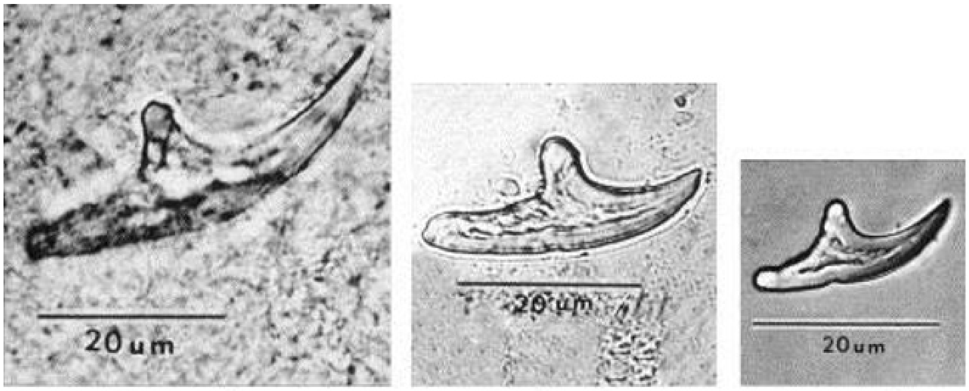


Figure 17.12 Polycystic hydatid disease. (Upper) From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. (Lower) Polycystic echinococcosis in the human liver: fatal case, from a resident of Panama (courtesy of Octavio Sousa, Centro de Investigación y Diagnóstico de Enfermedades Parasitarias, Facultad de Medicina, República de Panamá, from D'Alessandro A, Rausch RL, *Clin Microbiol Rev* **21**:380–401, 2008). doi:10.1128/9781555819002.ch17.f12



E. vogeli *E. oligarthrus* *E. granulosus*

Figure 17.13 Large rostellar hooks from protoscolices of *Echinococcus vogeli*, *E. oligarthrus*, and *E. granulosus* (left to right, all at the same magnification). (From D'Alessandro A, Rausch RL, *Clin Microbiol Rev* **21**:380–401, 2008.) doi:10.1128/9781555819002.ch17.f13

Ultrasound and CT showed a large, liquid-filled vesicle 27 by 32 mm, surrounded by a 3-mm-thick fibrotic capsule at the apex of the orbit.

Diagnosis

In 80% of the patients, the lesions were in the liver alone or in both the liver and other organs. In the remainder of the cases, they were located in the lung or other single sites. Patients were diagnosed by various imaging techniques (radiography, ultrasonography, CT scan). Serologic tests did not always confirm the infection; species identifications were based on morphological characteristics of the hooklets from protoscolices. Aspiration cytology has also been reported and can be very helpful in confirming the cause of the "mass." Another option is DNA analysis by PCR using protoscolices. In cases where the organism was identified to the species level, the ratio of *E. vogeli* to *E. oligarthrus* was approximately 10:1.

Prior to recognition of the parasitic etiology, in cases of polycystic disease, the intra-abdominal masses were erroneously considered to represent a variety of disorders, including hepatic tumor, abscess, cirrhosis or cholecystitis, gallbladder cancer, mesenteric tumor, and costal chondrosarcoma.

Increases of alkaline phosphatase, bilirubin, liver transaminases, and gamma globulin and diminished albumin and hemoglobin are common findings in cases of polycystic echinococcosis (47). Eosinophilia was reported in about 21% of cases, ranging from 9 to 28%, but it is not considered to have specific diagnostic value.

Treatment

Albendazole therapy has resulted in clinical improvement, with resolution or reduction in the size of the cysts. Because surgical resection is difficult at best, some physicians think that albendazole therapy should be tried prior to surgical intervention. However, a combination of surgery and albendazole therapy has also been advocated.

Epidemiology and Prevention

Human polycystic echinococcosis is now recognized as a distinct disease entity. Although the cestodes that cause polycystic hydatid disease are indigenous to the humid tropical forests in Central and northern South America, *E. oligarthrus* has now been found in North America (51). This is the first time that the adult worm has been recovered from a bobcat. The isolate was identified from San Fernando, Tamaulipas State, in northeast Mexico; this location is approximately 100 km south of Brownsville, TX. Apparently, infection risk exists in a larger geographic area than was previously suspected.

The paca is widely hunted and used for food in northern South America, and local people tend to feed the viscera of these animals to their dogs. Based on this common practice, it is assumed that the dog is probably the primary source of infection for humans in this and other areas of endemic infection.

Taenia (*Multiceps*) spp. (*Taenia multiceps*, *Taenia serialis*) (Coenurosis)

Although the adult tapeworms resemble *Taenia* spp., the larval form is a coenurus, a unilocular cyst with the same configuration as a cysticercus but containing multiple scolices. Human cases have been reported worldwide; however, identification to the species level is often very difficult if not impossible (Table 17.1).

Life Cycle and Morphology

The dog, wolf, and fox are the definitive hosts for *Taenia* (*Multiceps*) spp. (Fig. 17.14). Infection of the intermediate host (herbivores) occurs on ingestion of infective eggs from

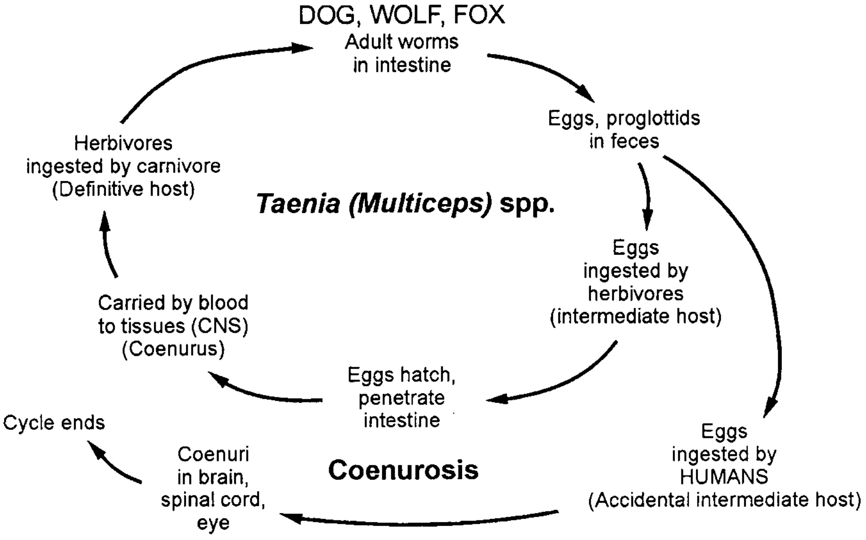

DOG, WOLF, FOX
Adult worms in intestine
Eggs, proglottids in feces
Herbivores ingested by carnivore (Definitive host)
Taenia (Multiceps) spp.
Eggs ingested by herbivores (intermediate host)
Carried by blood to tissues (CNS) (Coenurus)
Eggs hatch, penetrate intestine
Cycle ends
Coenuri in brain, spinal cord, eye
Coenurosis
Eggs ingested by HUMANS (Accidental intermediate host)

Figure 17.14 Life cycle of *Taenia* (*Multiceps*) spp. doi:10.1128/9781555819002.ch17.f14

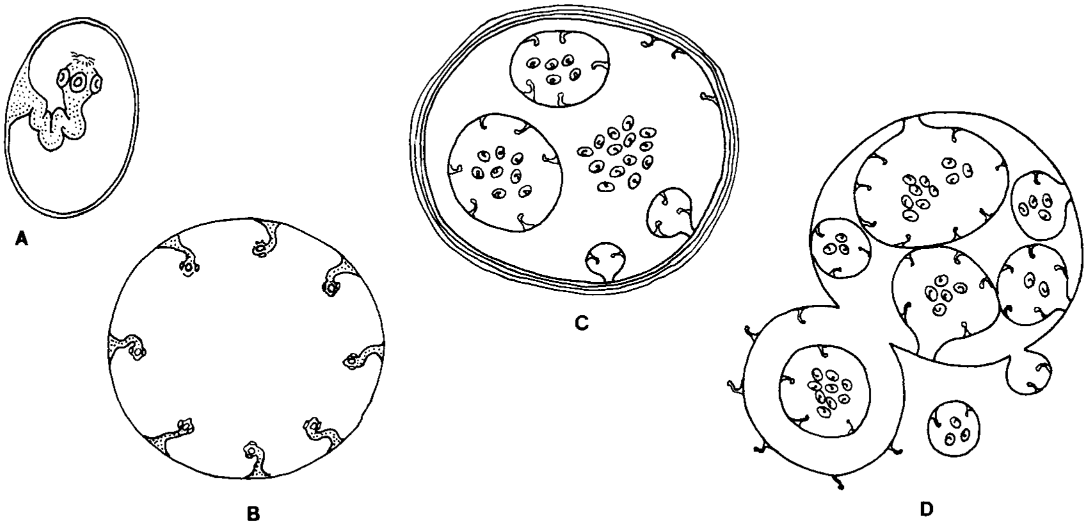
the adult tapeworm in the carnivore intestine. The oncosphere hatches in the intestine, penetrates the intestinal wall, and is carried via the bloodstream to various parts of the body, primarily the central nervous system. In sheep, one of the more common herbivore intermediate hosts, the presence of the coenurus in the central nervous system causes a disease known as gid (unstable gait or giddiness). When these infected herbivore tissues are ingested by a carnivore, each scolex within the coenurus has the potential to become an adult worm in the carnivore's intestine.

The coenurus structure is intermediate between that of a cysticercus with a single scolex and that of a hydatid cyst with both multiple protoscolices and daughter cysts. The coenurus contains multiple protoscolices but no daughter cysts (Fig.17.15 to 17.17).

Clinical Disease

In humans, fewer than 175 cases have been reported. Most coenuri have been located in the brain or spinal cord, but they can also be found in the subcutaneous tissues. Symptoms have been those associated with any space-occupying lesion, including headache, vomiting, paraplegia, hemiplegia, aphasia, seizures, and eye symptoms (if the coenurus is present in the eye).

Figure 17.15 Representative cysts. (A) Cysticercus; (B) coenurus; (C) hydatid; (D) multilocular hydatid. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch17.f15



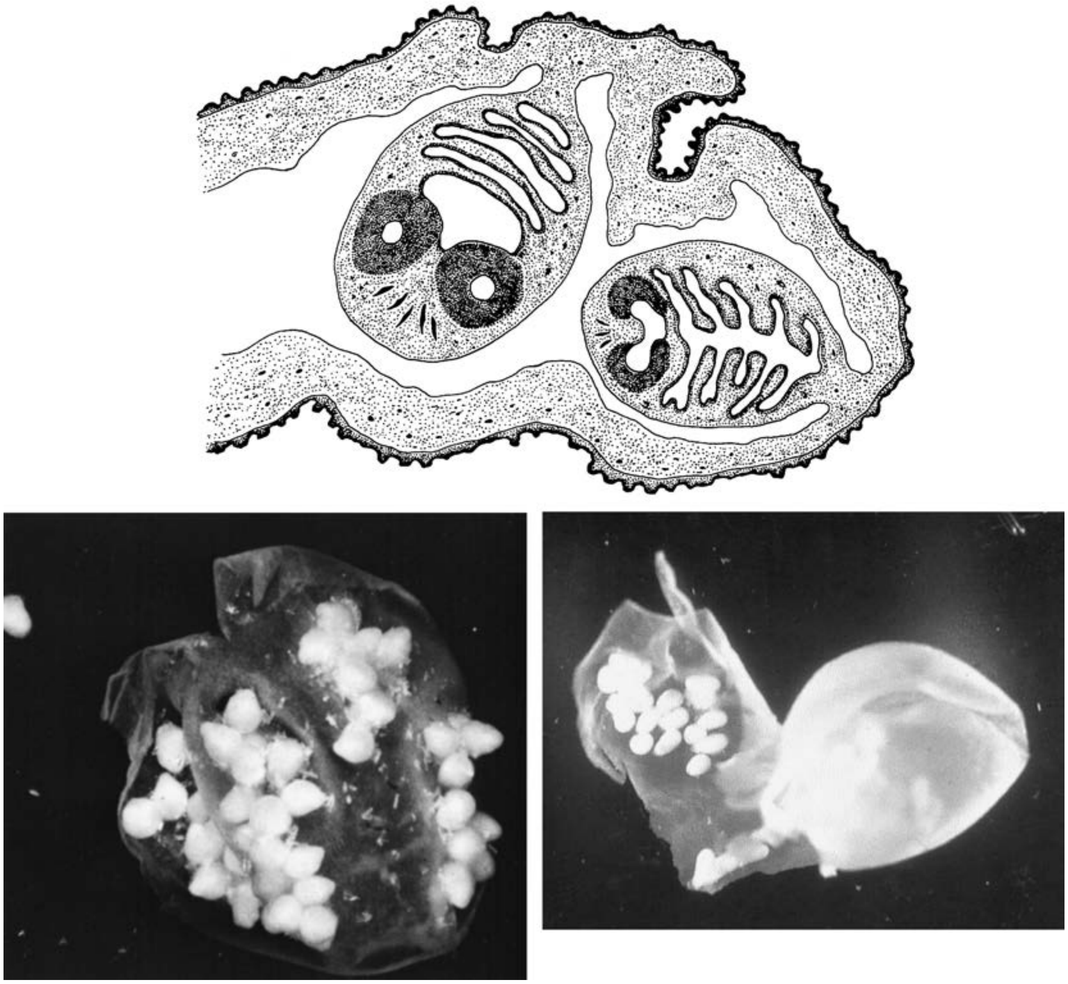


Figure 17.16 Coenurosis. (Upper) Diagram of cyst. (Illustration by Sharon Belkin.) (Lower) Actual cysts. (Left, Armed Forces Institute of Pathology photograph. Right, from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman; photograph courtesy J. Harper III and L. C. Marcus.) doi:10.1128/9781555819002.ch17.f16

Diagnosis

Diagnosis is based on gross and histologic examinations of the coenurus after surgical removal.

Treatment

Surgical removal, if possible, is recommended. Although praziquantel has been effective in killing these parasites, there is some concern about the marked inflammation which occurs as a result of treatment. Often albendazole is combined with praziquantel.

Epidemiology and Prevention

An understanding of the manner of transmission among carnivores and herbivores suggests the importance of proper disposal of carcasses of potentially infected herbivores. Also, good personal hygiene for those in close contact with dogs in areas of the world where herbivores (cattle, goats, and sheep) are raised is recommended. In 2009, the first successful field test of a practical vaccine against *T. multiceps* in sheep was published; considering the high degree of success achieved, this approach could lead to routine use in areas at particular risk such as Sardinia (52).

Spirometra mansonoides and *Diphyllobothrium* spp. (Sparganosis)

The larval forms (spargana) of *Spirometra mansonoides* and *Diphyllobothrium* spp. were first recognized in humans from tissues removed at autopsy. In areas where infections are endemic, similar forms can be found in the

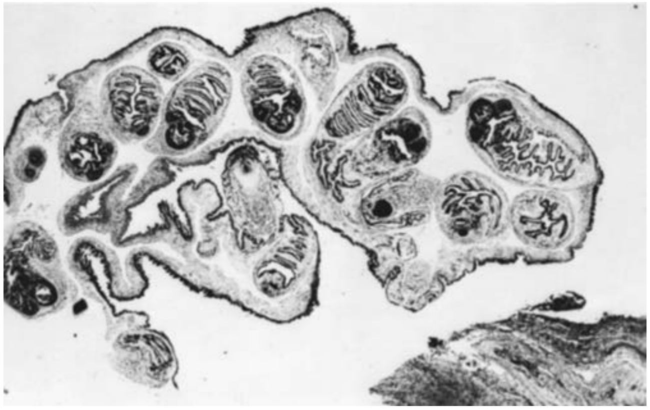


Figure 17.17 Coenurosis: cross section of cyst. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch17.f17

subcutaneous connective tissue and between the muscles in frogs, lizards, snakes, birds, and certain mammals. When these spargana are fed to the definitive hosts (dogs and cats), adult tapeworms of several related species of *Spirometra* develop. The general name for these spargana is *Spirometra mansonoides*. Although cases have been reported worldwide, sparganosis is most common in China, Japan, and Southeast Asia (Table 17.1).

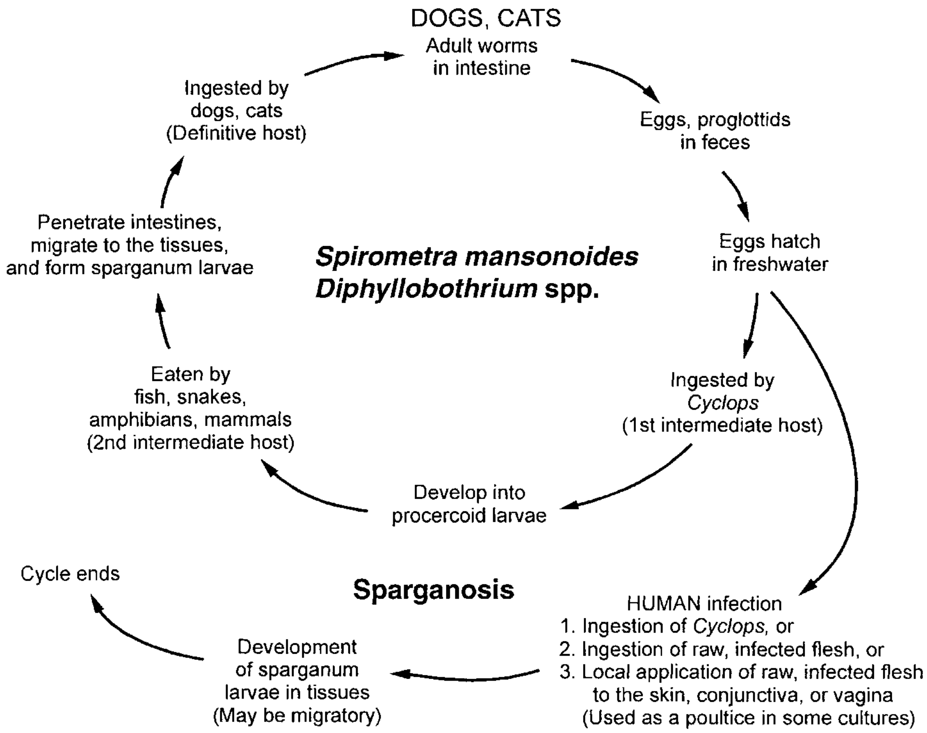
Life Cycle and Morphology

Spargana. Eggs passed from adult tapeworms in the dog or cat intestine hatch in freshwater and are ingested by *Cyclops* spp., in which they develop into the procercoid larva. When the *Cyclops* spp. are eaten by a second intermediate host (fish, snakes, amphibians, or mammals), the procercoid larva penetrates the intestinal wall, migrates to the tissues, and develops into the larva (the sparganum). On ingestion by a dog or cat, the sparganum develops into the adult tapeworm, thus completing the life cycle (Fig. 17.18).

Human infection can occur from (i) ingestion of infected *Cyclops* spp. (the procercoid stage penetrates the intestinal wall, migrates into tissues, and develops into the sparganum), (ii) ingestion of raw infected flesh of one of the second intermediate hosts (fish, snakes, amphibians, and mammals), or (iii) local application of raw, infected flesh of one of the second intermediate hosts to the skin, conjunctiva, or vagina (the spargana migrate from vertebrate host tissues into human tissue). The spargana are white, ribbonlike worms measuring from a few millimeters to several centimeters long. Human infections with spargana are classified into two forms, nonproliferative sparganosis and proliferative sparganosis (aberrant spargana).

The sparganum is a wrinkled, white, ribbon-shaped organism, 3 mm in width and up to 30 cm long. It is a

Figure 17.18 Life cycle of *Spirometra mansonoides* and *Diphyllobothrium* spp. (sparganosis).
doi:10.1128/9781555819002.ch17.f18


DOGS, CATS
Adult worms in intestine
Eggs, proglottids in feces
Eggs hatch in freshwater
Ingested by *Cyclops* (1st intermediate host)
Develop into procercoid larvae
Eaten by fish, snakes, amphibians, mammals (2nd intermediate host)
Penetrate intestines, migrate to the tissues, and form sparganum larvae
Ingested by dogs, cats (Definitive host)
Spirometra mansonoides
Diphyllobothrium spp.
Sparganosis
HUMAN infection
1. Ingestion of *Cyclops*, or
2. Ingestion of raw, infected flesh, or
3. Local application of raw, infected flesh to the skin, conjunctiva, or vagina (Used as a poultice in some cultures)
Development of sparganum larvae in tissues (May be migratory)
Cycle ends

solid-bodied larva that has no bladder and has bothrids (two longitudinal grooves) at the anterior end instead of suckers (similar to the scolex of *Diphyllobothrium latum*). The larva has an unsegmented strobila 20 to 30 cm in length, bundles of longitudinal muscle fibers scattered throughout the mesenchyme, and a thick tegument (Fig. 17.19).

There are several species of *Spirometra*, including *S. mansoni*, *S. mansonoides*, *S. erinacei*, *S. ranarum*, *S. decipiens*, *S. houghtoni*, and *S. proliferum*. The larvae are often referred to by the generic name *Sparganum*, because this stage was previously thought to represent a separate genus. *S. mansoni* is the predominant species in Asia (highest endemicity in Korea and Japan), while *S. mansonoides* is the predominant species in the United States. Cases of *S. proliferum* have been reported in Japan, the United States, Venezuela, and Paraguay.

Aberrant Spargana. *S. proliferum* is a rare tapeworm larva that grows by continuous branching and budding. The normal tissue organization and symmetry are no longer visible. The lesion gradually expands and may ulcerate to form subcutaneous or cutaneous lesions. The adult form of this organism is unknown.

Clinical Disease

Spargana. Most patients have slowly growing, tender, subcutaneous nodules. These nodules may also be migratory. Ocular sparganosis is accompanied by pruritus, pain, lacrimation, and edematous swelling of the eyelid (which may look much like Romaña's sign in Chagas' disease). Depending on the location of the sparganum, there may be elephantiasis (lymph channels), peritonitis (intestinal perforation), or brain abscess. Most patients have leukocytosis and eosinophilia. The sparganum has been reported to live for more than 9 years. In a study from Thailand, 17 cases were ocular, 10 were subcutaneous, 5 involved the central nervous system, 1 was auricular, 1 was pulmonary, 1 was intraosseous, and 1 was intraperitoneal. Risk factors included a history of drinking impure water, eating frog or snake meat, or using frog or snake meat as a poultice; some patients had multiple risk factors. Most of these patients presented with superficial ocular mass lesions (53). Sparganosis can cause intestinal obstruction, and the parasite has been recovered from the wall of the intestine, breast, scrotum, epididymis, ureter, urinary bladder, abdominal cavity, heart, and lung (Fig. 17.19).

Ocular sparganosis causes intense reactions, with periorbital edema. This infection may result in blindness as the parasite migrates to the conjunctiva and enters the orbit. In the orbital tissues, the larva is located in the posterior pole, producing inflammation that results in exophthalmia (bulging of the eye) and lagophthalmia (inability to close the eyelids completely) and can result in ulceration

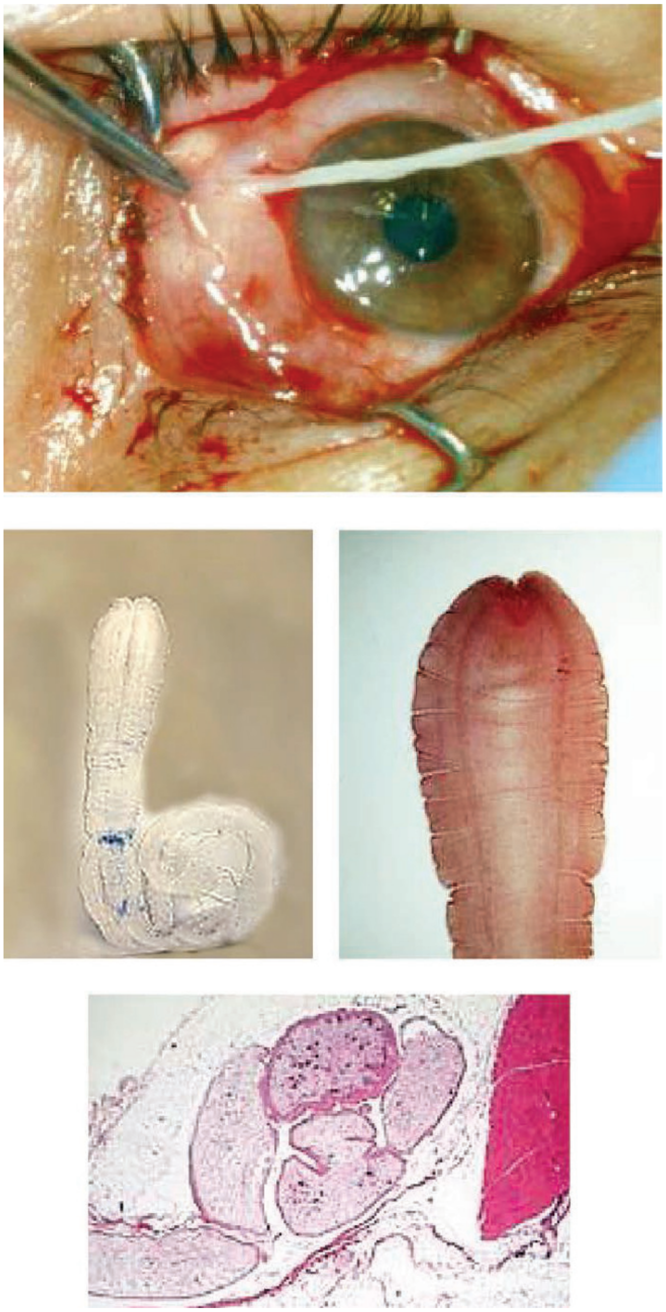


Figure 17.19 Sparganosis. (Top) Surgical removal of a nodular mass and appearance of an inert, whitish, flat structure (courtesy *Rev Inst Med Trop S Paulo*, http://dx.doi.org/10.1590/S0036-46652011000100009). (Middle, left) Sparganum (courtesy of CDC Public Health Image Library); (right) scolex of sparganus (courtesy of Tai Soon Yong, Web Atlas of Medical Parasitology). (Bottom) Muscle. Within the loose fibroadipose tissue adjacent to skeletal muscle, there are multiple profiles of an immature tapeworm (pleurocercoid), composed of loose mesenchyme bearing abundant oval-shaped calcified bodies (calcarious corpuscles). (Courtesy of the Armed Forces Institute of Pathology.)
doi:10.1128/9781555819002.ch17.f19

of the cornea. There is often intense pain, irritation, excessive lacrimation, and marked swelling of the eyelids.

Cerebral sparganosis symptoms include seizures, fatigue, confusion, headaches, memory loss, coma, fever, paresthesias, hemiparesis, motor weakness, and other central nervous system symptoms. The infection generally involves the cerebral hemispheres, especially the frontoparietal lobes, and in some cases extends to the cerebellum. The disease may appear as a massive cerebral hemorrhage.

Aberrant Spargana. In the few documented cases of *S. proliferum* infections, thousands of spargana have been recovered in subcutaneous tissues, intermuscular fasciae, intestinal wall, mesentery, kidneys, lungs, heart, brain, and bone. Proliferative sparganosis begins with a subcutaneous tumor in the thigh, shoulder, or neck and eventually spreads to other parts of the skin, the muscles, and the internal organs such as the lungs, abdomen, and brain. Nodules may open because of ulceration or scarification. Infection progresses over 5 to 25 years and is fatal in all reported cases.

Diagnosis

Spargana. Confirming the site of the lesion using CT or MRI is helpful in diagnosing sparganosis. However, removal of the lesion and identification of the tapeworm is often required for definitive diagnosis. Usually, diagnosis is not made until drainage of the lesion, surgical removal of the worms, and gross and/or microscopic examination of the tissue have been performed. The more elongate, wormlike structure of the sparganum can usually be distinguished from the bladder structure of a cysticercus or coenurus, since the last two have suckers and hooklets, both of which are lacking in a sparganum. The parasites are usually recovered alive and are approximately several centimeters in length (54). They are glistening white and opaque, resemble narrow tapeworm proglottids, and are motile, similar to the motility seen with a *Taenia saginata* proglottid.

Aberrant Spargana. This rare form of sparganosis can be diagnosed by histologic examination. The tissue may appear to have many separate worms; however, each cross section probably represents a continuous branching worm.

KEY POINTS—LABORATORY DIAGNOSIS

***Spirometra mansonoides* and *Diphyllobothrium* spp. (Sparganosis)**

1. The diagnosis is usually based on the identification of the worm after surgical removal.
2. The absence of suckers and hooklets will usually differentiate spargana from a cysticercus or coenurus.

Treatment

Surgical removal is the recommended approach. Drug therapy is usually not effective; however, treatment with praziquantel has been used in the past. Triclabendazole, a combination of praziquantel and albendazole, can also be used, with surgical removal of the parasites. In the proliferating type of infection, surgical removal is very difficult if not impossible.

Epidemiology and Prevention

Considering the possible routes of human infection, all drinking water in areas of endemicity should be boiled or filtered to prevent accidental ingestion of *Cyclops* spp. The ingestion of raw tadpole, frog, snake, fowl, or mammalian flesh should be avoided in these areas. Educational information on the dangers of local application of raw, infected animal flesh (poulticing) to humans, particularly those with ulcerated lesions, should be disseminated.

Although sparganosis is more common in southeast Asia, it is infrequently reported from other areas of the world. A recent publication reports 37 cases from Ethiopia (3 cases) and South Sudan (34 cases). As a function of *Dracunculus* eradication efforts in the few remaining areas of endemicity, all suspect cases are investigated and worms are examined for confirmation. During the last few years of this confirmation process, 37 of these helminths were microscopically identified as spargana, not *Dracunculus*. Identification was based on several criteria including the presence of calcareous corpuscles in the somatic matrix and molecular testing. Like dracunculiasis, this series of cases confirms the risks of drinking unprotected water, eating poorly cooked animals that serve as paratenic hosts, and/or using raw, infected flesh of one of the second intermediate hosts as a poultice (84).

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18

Intestinal Trematodes

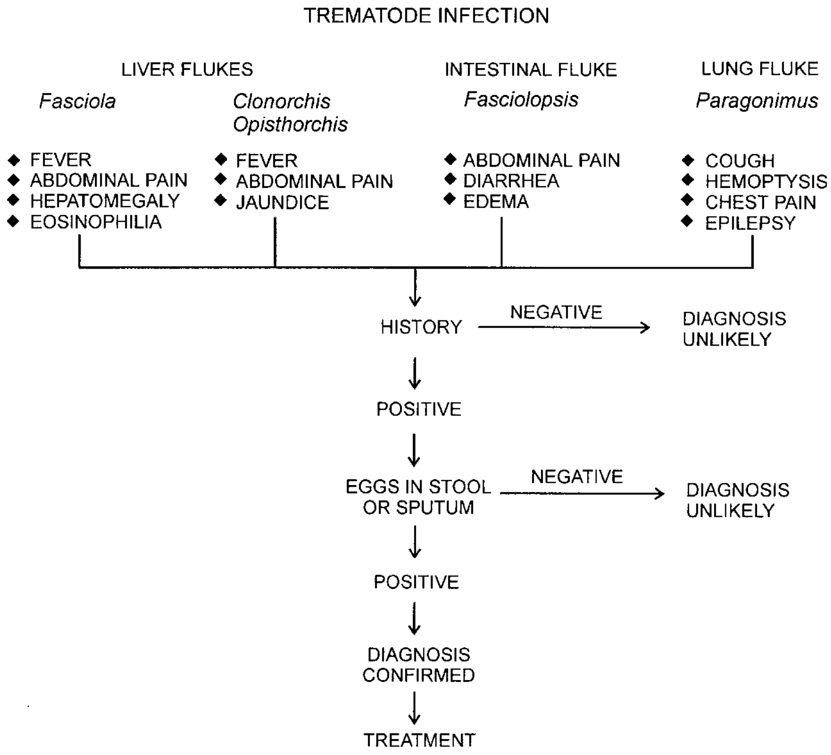
- *Fasciolopsis buski*
- *Echinostoma ilocanum*
- *Heterophyes heterophyes*
- *Metagonimus yokogawai*
- *Gastrodiscoides hominis*

The intestinal trematodes, or flukes, are parasites of vertebrates; they are dorsoventrally flattened and hermaphroditic and require one or more intermediate hosts. The adult worms vary in size from the barely visible (*Heterophyes heterophyes*) to the very large (*Fasciolopsis buski*). To complete the life cycle, specific species of intermediate hosts must be available for trematode development. All of the intestinal trematodes require a freshwater snail to serve as an intermediate host. These infections are food borne and are emerging as a major public health problem, with more than 50 million people being infected and an estimated 750 million at risk for these infections (Algorithm 18.1) (1). The true economic impact of these infections is difficult to assess. Intestinal trematode infections can be found in Southeast Asia, the Far East, the Middle East, and North Africa. For example, a total of 70 species (14 families and 36 genera) of intestinal flukes have been reported to have been isolated from humans (2).

Eggs deposited by the adult worms are passed to the outside in the feces (Fig. 18.1). All intestinal trematode eggs have an operculum, or "lid," from which the miracidium larva can escape. Eggs hatch in freshwater, and these larvae must find their way into the snail (intermediate host) through penetration of the snail tissues; in some cases, the snail ingests the eggs before they hatch. Over a period of weeks, a series of developmental stages occur within the snail, eventually producing cercariae, which are released from the snail. Typical features of cercariae include the tail, mouth, gut, suckers, flame cells, and glands. These cercariae then encyst on aquatic plant material or encyst in the tissues of freshwater mollusks or fish and become metacercariae; infectivity lasts from 1 h to several months. When the plants or mollusks are ingested raw, the metacercariae then infect the human host, where they excyst in the small intestine and develop into mature adult trematodes within the intestinal tract.

The trematode body surface is covered by a syncytial epithelium, the tegument. The tegument is involved in nutrient absorption, synthesis, secretion, and osmoregulation and has sensory functions. Moreover, the tegument protects the parasite from the immune system of the host. Further typical characteristics of trematodes are the presence of an oral sucker, often a ventral sucker, and a lack of respiratory and circulatory systems.

doi:10.1128/9781555819002.ch18


TREMATODE INFECTION
LIVER FLUKES
Fasciola
◆ FEVER
◆ ABDOMINAL PAIN
◆ HEPATOMEGALY
◆ EOSINOPHILIA
Clonorchis
Opisthorchis
◆ FEVER
◆ ABDOMINAL PAIN
◆ JAUNDICE
INTESTINAL FLUKE
Fasciolopsis
◆ ABDOMINAL PAIN
◆ DIARRHEA
◆ EDEMA
LUNG FLUKE
Paragonimus
◆ COUGH
◆ HEMOPTYSIS
◆ CHEST PAIN
◆ EPILEPSY
HISTORY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
EGGS IN STOOL OR SPUTUM
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
DIAGNOSIS CONFIRMED
TREATMENT

Algorithm 18.1 Trematode infection.
doi:10.1128/9781555819002.ch18.Alg18.1

Adult worms vary in shape and size and can be distinguished from one another (Table 18.1; Fig. 18.2). The adult worms are hermaphroditic, containing both male and female reproductive systems.

Fasciolopsis buski

Fasciolopsiasis was first noted by Busk in 1843, when worms were detected in the duodenum of a deceased East Indian sailor (3). *F. buski* has also been known as the giant intestinal fluke and is one of the largest parasites to infect humans, measuring 20 to 75 mm in length, 8 to 20 mm in width, and 0.5 to 3 mm in thickness.

Life Cycle and Morphology

The adult worms live in the small intestine of pigs and humans, where the worms lay unembryonated eggs that are then passed from the intestinal lumen with the feces. The eggs are ellipsoidal, operculate, and yellow-brown. They measure 130 to 140 μm by 80 to 85 μm, with the operculum found at the more pointed end of the transparent eggshell (Fig. 18.3, 18.4, 18.5, and 18.6; Table 18.1). Depending on the temperature, the

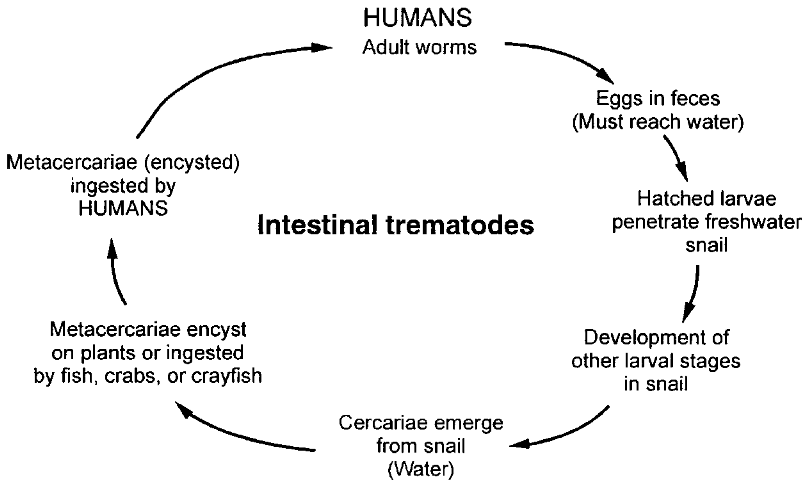

HUMANS
Adult worms
Eggs in feces
(Must reach water)
Hatched larvae
penetrate freshwater
snail
Development of
other larval stages
in snail
Cercariae emerge
from snail
(Water)
Metacercariae encyst
on plants or ingested
by fish, crabs, or crayfish
Metacercariae (encysted)
ingested by
HUMANS
Intestinal trematodes

Figure 18.1 Life cycle of intestinal trematodes.
doi:10.1128/9781555819002.ch18.f1

TABLE 18.1 Characteristics of intestinal trematodes

Species	Distribution	Agent of infection	Reservoir host	Egg		
				Size (µm)	Morphology	Comments
Fasciolopsis buski	Far East	Water chestnut, bamboo shoots, water caltrop	Dogs, pigs, rabbits	130–140 by 80–85	Unembryonated operculated	The less mature the egg, the more difficult it may be to see the actual operculum—it blends into the shell outline, and the "breaks" in the shell may be difficult to identify. This egg has no opercular shoulders, and so it is difficult to see where the operculum "breaks" in the shell actually occur.
Echinostoma ilocanum	Far East	Freshwater fish, frogs, mussels, snails, snakes, tadpoles	Rats, dogs	86–116 by 59–69	Unembryonated operculated	Although *E. ilocanum* eggs are considerably smaller than *F. buski* eggs, some strains produce eggs that overlap in size; the operculum may be difficult to see (as indicated above for *F. buski*).
Heterophyes heterophyes	Far East, Middle East	Freshwater fish	Fish-eating mammals	27–30 by 15–17	Embryonated operculated with very subtle opercular shoulders; no "seated" operculum	These eggs have very inconspicuous opercular shoulders and, unlike *C. sinensis*, lack the "seated" operculum and knob at the abopercular end. *H. heterophyes* eggs are some of the smallest trematode eggs seen and may be missed using the low power of the microscope; high dry power may be required for identification.
Metagonimus yokogawai	Far East, former USSR, Israel, Spain	Freshwater fish	Fish-eating mammals	26–28 by 15–17	Embryonated operculated with minimal opercular shoulders; no "seated" operculum	These eggs have inconspicuous opercular shoulders but a more obvious operculum than *H. heterophyes* eggs and are some of the smallest trematode eggs. They do not really have the "seated" operculum and knob at the abopercular end like those seen in *C. sinensis* eggs. They may be missed using the low power of the microscope, and high dry power may be required for identification.
Gastrodiscoides hominis	Far East, Middle East, former USSR	Freshwater fish	Pigs, deer mice, rats	60–70 by 150	Unembryonated operculated	The egg of *G. hominis* tends to be more slender than that of *F. buski*, but they are very much alike; this egg has no opercular shoulders, and so it is difficult to see where the operculum "breaks" in the shell actually occur.

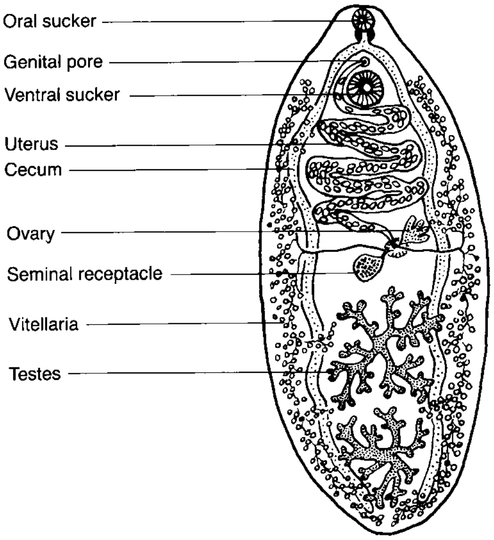
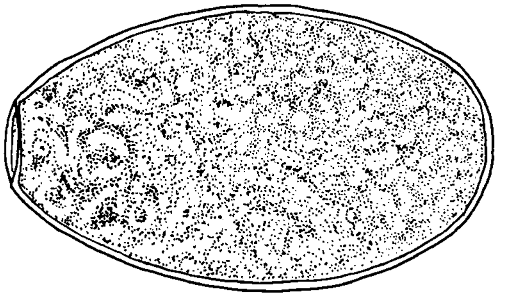


Figure 18.2 Diagram of a general intestinal/liver trematode. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch18.f2

eggs embryonate within 3 to 7 weeks. Once in the water, the mature miracidium hatches from the egg and tries to locate the appropriate snail species to infect (*Segmentina* and *Hippeutis* spp.). Once the miracidium has penetrated the soft tissues of the snail (first intermediate host), it begins to develop into a first-generation sporocyst. The sporocyst is an elongated sac, without distinct internal structures, in which germ balls proliferate. These germ balls develop into rediae that contain a mouth, pharynx, blind cecum, and birth pore. Within the rediae, the germ balls again proliferate, developing into cercariae. Unlike some of the other intestinal trematodes, *Fasciolopsis* rediae develop a second generation of rediae before forming cercariae. On reaching maturity, the cercariae escape from the snail host into the water. The cercariae encyst on water plants such as water caltrops, water chestnuts, and water bamboo, where they develop into metacercariae in approximately 4 weeks (Fig. 18.7). Humans become infected by ingesting the raw or undercooked plants containing the metacercariae. The metacercariae excyst, attach to the duodenal or jejunal mucosa, and develop into adult worms within 3 months (Fig. 18.6). The adult life span seldom exceeds 6 months to a year (3).

Figure 18.3 *Fasciolopsis buski* egg. (Illustration by Nobuko Kitamura.) doi:10.1128/9781555819002.ch18.f3



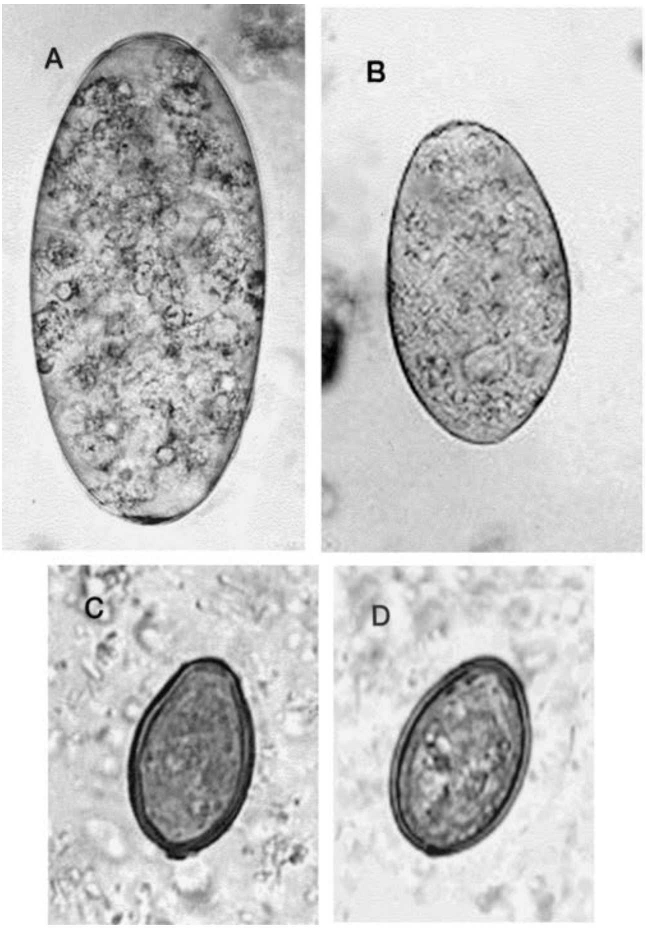


Figure 18.4 Intestinal trematode eggs. (A) *Fasciolopsis buski*; (B) *Echinostoma ilocanum*; (C) *Heterophyes heterophyes*; (D) *Metagonimus yokogawai*. Note that the eggs in panels C and D are shown at a higher magnification to demonstrate very minor differences; the operculum of *H. heterophyes* is less pronounced than that of *Clonorchis sinensis* (see chapter 19), and the operculum outline of *M. yokogawai* is the least obvious of the three small trematode eggs that are often confused (*C. sinensis*, *H. heterophyes*, and *M. yokogawai*). doi:10.1128/9781555819002.ch18.f4

F. buski is the largest of the intestinal trematodes and attaches to the duodenal and jejunal walls (Fig. 18.6). The adult worms are fleshy, somewhat pale, and elongate-ovoid and have no cephalic cone structures like that seen in the liver fluke, *Fasciola hepatica*. The large egg tends to be yellowish brown, has a clear, thin shell with a small operculum but no opercular shoulders, and is nearly identical to that of *F. hepatica* and *F. gigantica*. The less mature the egg, the more difficult it is to see the operculum "breaks" in the shell outline.

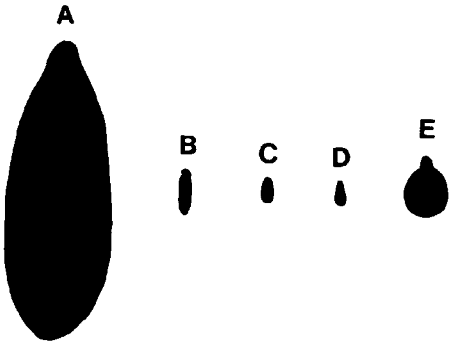
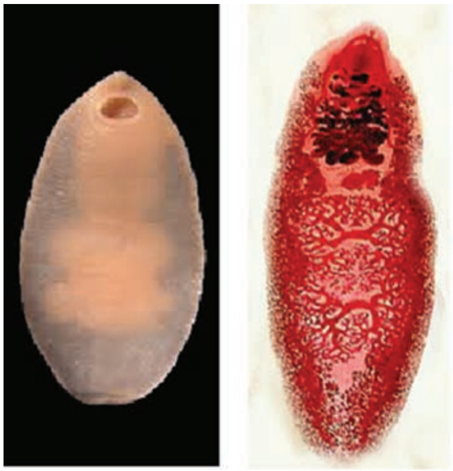


Figure 18.5 Outline of the sizes of adult intestinal trematodes. (A) *Fasciolopsis buski*; (B) *Echinostoma ilocanum*; (C) *Heterophyes heterophyes*; (D) *Metagonimus yokogawai*; (E) *Gastrodiscoides hominis*. doi:10.1128/9781555819002.ch18.f5

Clinical Disease

Regarding intestinal fluke infections, school-age children were found to be at the highest risk of infection and infection intensity, as documented previously for *F. buski* (4). In light infections, the adult worms inhabit the duodenum and jejunum; in heavy infections, they may be found in the stomach and most of the intestinal tract. The attachment of worms to the mucosal wall produces local inflammation with hypersecretion of mucus, hemorrhage, ulceration, and possible abscess formation. In heavy infections, the worms may cause bowel obstruction, acute ileus, and absorption of toxic or allergic worm metabolites, producing general edema and ascites. Edema of the face may occur as a result of hypoalbuminemia secondary to malabsorption or protein-losing enteropathy. Occasionally, vitamin B_{12} absorption is impaired, with resulting low vitamin B_{12} levels. A marked eosinophilia and leukocytosis are commonly seen. Few symptoms are associated with light infections, but in heavier infections, the patient may experience abdominal pain and diarrhea. In heavy infections, the stools are profuse and yellow-green and contain increased amounts of undigested food, suggesting a malabsorption process. The symptoms may be confused with those of giardiasis or peptic ulcer or with other causes of bowel obstruction. Depending on the worm burden, the infection can be associated with severe cachexia and prostration and can lead to death (5).

Figure 18.6 *Fasciolopsis buski* adult worms. (Left) Unpreserved adult worm: fleshy, pale, elongate-ovoid with no cephalic cone as seen in *F. hepatica*; the adult *F. buski* worms measure 20 to 75 mm in length by 8 to 20 mm in width, and 0.5 to 3 mm in thickness. (Right) Stained and flattened adult worm: from anterior to posterior characterized by a large ventral sucker, coiled uterus, branched ovary, and two branched testes filling most of the posterior section of the worm; the lateral fields are filled with vitellaria. doi:10.1128/9781555819002.ch18.f6



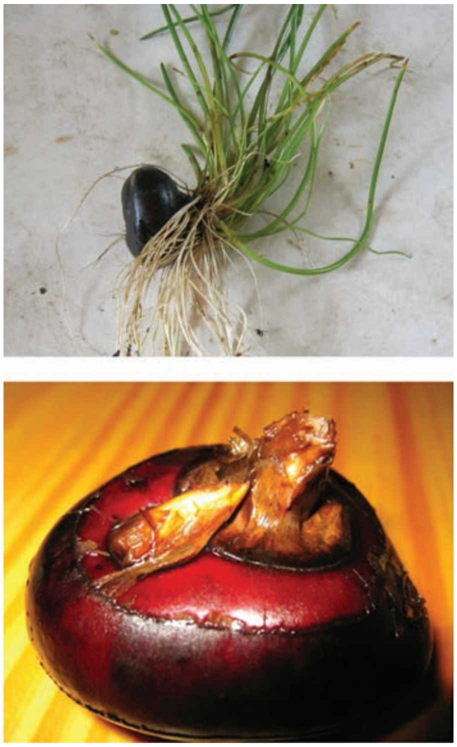


Figure 18.7 *Eleocharis dulcis*, water chestnut. (Upper) Plant structure (courtesy of Mass Spectrum Botanicals). (Lower) Chestnut (courtesy of Joscha Feth at the German Wikipedia project—released into the public domain). doi:10.1128/9781555819002.ch18.f7

Diagnosis

The diagnosis is suggested by the clinical features in areas where infections are endemic and is confirmed by detecting the eggs in the stool. The zinc sulfate flotation concentration is not recommended for concentrating trematode eggs because of the operculum and the fragility of the eggshell. The high specific gravity of the zinc sulfate causes the operculum to "pop" open, the eggshell fills with fluid, and the egg sinks to the bottom of the tube, where it may appear to be very distorted. Therefore, when this concentration procedure is used, both the upper flotation layer and the sediment must be examined. The formalin-ethyl acetate sedimentation concentration is recommended for routine fecal concentration. The sedimented material can be examined with or without iodine. The eggs of *Echinostoma ilocanum*, *Fasciola hepatica*, *F. buski*, and *Gastrodiscoides hominis* are similar in size and shape; therefore, an exact identification cannot be made from examining the eggs. It is possible to detect adult worms in the stool in heavy infections when they lose their ability to remain attached to the intestinal mucosa.

Other methods available include Kato-Katz thick smears or Stoll's dilution egg count method. Also, excellent results have been reported with the FLOTAC technique, a multivalent fecal egg count technique (6). It is important to remember that trematode species-specific diagnosis on the basis of egg morphology is difficult.

KEY POINTS—LABORATORY DIAGNOSIS

Fasciolopsis buski

1. The eggs are found in the stool; rarely, adult worms are found during heavy infections.
2. The eggs of *F. buski*, *E. ilocanum*, *G. hominis*, *F. hepatica*, and *F. gigantica* appear almost identical and are very difficult to differentiate from one another. When passed, the eggs do not contain mature larvae but instead contain undifferentiated embryos.
3. The formalin-ethyl acetate sedimentation concentration is recommended for egg recovery; operculated eggs do not float in the zinc sulfate flotation concentration method.
4. The less mature the egg, the more difficult it may be to see the actual operculum—it blends into the shell outline, and the "breaks" in the shell may be difficult to identify. This egg has no opercular shoulders; therefore, it is difficult to see where the operculum "breaks" in the shell actually occur.

Treatment

Although different drugs have been used for therapy, the drug of choice is praziquantel, an isoquinoline derivative that is administered orally (7). The drug is well tolerated; however, there may be some side effects, including abdominal pain, headache, dizziness, nausea, drowsiness, pruritus, and myalgia. These side effects usually disappear within 48 h but may be more pronounced in heavily infected individuals. The drug is administered as a single 15-mg/kg dose after the evening meal or before going to bed. A second regimen consists of 75 mg/kg/day in three doses for 1 day. Apparently, children tend to tolerate the drug better than adults. Contraindications for use include ocular cysticercosis, cerebral paragonimiasis, pregnancy, and physically demanding tasks where dizziness or drowsiness might put the patient at risk.

An alternative drug is niclosamide (Niclocide), a salicylamide derivative; alcohol should be avoided during treatment. The drug may be given for 1 or 2 days at 40 mg/kg/day (up to a maximum of 4 g) (8). This drug is minimally absorbed from the gastrointestinal tract, and side effects include nausea, vomiting, diarrhea, and abdominal pain.

Epidemiology and Prevention

F. buski reservoir hosts include dogs, pigs, and rabbits. The infection is commonly found in Bangladesh, Cambodia, central and southern China, India, Indonesia, Laos, Malaysia, Pakistan, Taiwan, Thailand, and Vietnam; it has also been reported from Japan. Drainage of farm waste, use of manure for cultivation, and defecation in or near ponds or lakes that contain snails from the family *Planorbidae* (*Gyraulus*, *Polypylis*, *Segmentina*, or *Hippeutis* spp.), with water plants acting as vectors, allow the life cycle of the worm to continue. Metacercariae encyst on freshwater vegetation, such as water chestnuts, bamboo shoots, or water caltrops, and the infection is acquired when these infested plants are consumed raw or the outer coat is peeled off the nut with the teeth, resulting in accidental ingestion.

To prevent the infection, plants should be cooked or immersed in boiling water for a few seconds before they are eaten or peeled. In areas of endemicity, the use of unsterilized night soil for fertilizer should be prohibited. If these safeguards were used, the risk of infection would be considerably decreased.

Fasciolopsiasis remains a public health problem despite changes in eating habits, agricultural practices, health education, industrialization, and environmental approaches. The disease occurs focally and is most prevalent in school-age children. In areas of endemicity, the prevalence of infection in children ranges from 57% in mainland China to 25% in Taiwan and from 50% in Bangladesh and 60% in India to 10% in Thailand. Control programs are not fully successful because of long-standing traditions of eating raw aquatic plants and using untreated water. Control measures are also impacted by social and economic factors, an expanding free-food market, a lack of sufficient food inspection and sanitation, and declining economic conditions (9, 10).

Echinostoma ilocanum

A number of species of echinostomes have been reported to infect humans, including *E. hortense*, *E. ilocanum*, *E. macrorchis*, *E. perforiatum*, and *E. revolutum*. Most of the species are found in Asian countries; *E. ilocanum* is the most important species. The infection was first noted by Garrison in 1907 (8). *Echinostoma* flukes are distinguishable from other trematodes by their distinctive horseshoe-shaped collar of spines on the lateral and dorsal sides of their oral sucker.

Life Cycle and Morphology

Adult worms are attached to the mucosal wall of the small intestine and lay eggs, which are passed from the intestinal lumen in the feces. The eggs are immature, ellipsoidal, yellow-brown, and operculated. They measure 86 to 116 μm by 58 to 69 μm (Table 18.1; Fig. 18.4). The miracidia take 1 to 2 weeks to mature in the environment before they hatch from the eggs and infect the snail intermediate host. Two or more generations of rediae are produced before cercariae develop. These cercariae encyst in freshwater mollusks, and humans are infected by eating these mollusks raw rather than cooked. The metacercariae hatch in the intestine, attach to the mucosal wall, and develop into mature adult worms.

The adults are less than 1 cm long and 0.2 cm wide and are reddish gray (Fig. 18.5). The anterior end of the adult worm has a circumoral disk with a crown of spines surrounding the oral sucker (Fig. 18.8).

Clinical Disease

With light infections, the patient may be asymptomatic and the adult worms cause only minor problems other than localized inflammation. In heavy infections, the worms can produce catarrhal inflammation and mild ulceration and the patient may experience diarrhea and abdominal pain.

Diagnosis

Eggs of *E. ilocanum* can be detected in the stool. They are the same size (some strains) and shape as the eggs of *F. hepatica* and *F. buski*. Because of the overlap in size range, exact identification cannot be made from the eggs; therefore, the diagnosis may have to be made on the basis of patient history or clinical findings.

KEY POINTS—LABORATORY DIAGNOSIS

Echinostoma ilocanum

1. The eggs are found in the stool; rarely, adult worms are found during heavy infections.
2. The eggs of *E. ilocanum*, *G. hominis*, *F. buski*, *F. hepatica*, and *F. gigantica* appear almost identical and are very difficult to differentiate from one another. When passed, the eggs do not contain mature larvae but instead contain undifferentiated embryos.
3. The formalin-ethyl acetate sedimentation concentration is recommended for egg recovery; operculated eggs do not float in the zinc sulfate flotation concentration method.
4. The less mature the egg, the more difficult it may be to see the actual operculum—it blends into the shell outline, and the "breaks" in the shell may be difficult to identify. This egg has no opercular shoulders; therefore, it is difficult to see where the operculum "breaks" in the shell actually occur.

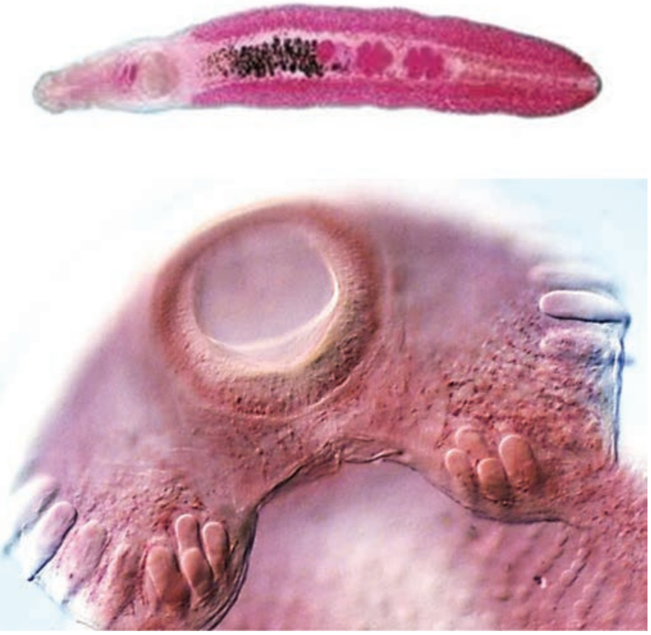
Treatment

Praziquantel is the drug of choice and can be given in a single dose of 40 mg/kg at bedtime. Another option would be albendazole at 400 mg twice a day for 3 days.

Epidemiology and Prevention

Information concerning *E. ilocanum* infections and epidemiology is limited. Both rats and dogs have been found to be infected in areas where infections are endemic. The infection has been reported from the Celebes, China, India, Indonesia, Japan, Korea, Malaysia, the Philippines, Russia, Taiwan, and Thailand. In some areas in northeastern Thailand, the infection rate may be as high as 50%. In areas of the Philippines with high levels of endemic infection (Luzon, Mindanao, and Leyte), local infection rates from 1 to 44% have been reported

Figure 18.8 *Echinostoma ilocanum* adult worms. (Upper) Stained adult worm characterized by having a circumoral disk with a crown of spines surrounding the small oral sucker. (Lower) Enlarged image of the crown of spines (surrounding the oral sucker) and the large ventral sucker. The living worm is reddish gray and measures 2.5 to 6.5 mm in length by 1 to 1.35 mm in width. (Courtesy of www.k-state.edu.) doi:10.1128/9781555819002.ch18.f8



(8). In China, the infection appears to be limited to dogs, with an infection rate of 14% being reported. The miracidia must infect a susceptible species of snail from the family *Planorbidae* (*Gyraulus* or *Hippeutis* spp.). The cercariae can encyst in a number of freshwater mollusks, particularly snails, which, if eaten raw, will allow the metacercariae to excyst in the intestinal tract and develop to adult worms. The infection can be prevented in areas of endemicity by restricting the use of night soil for fertilizer and eating cooked rather than raw mollusks. The disease is most prevalent in those areas where snails, fish, and other possible intermediate hosts are eaten raw.

Human infections with other echinostome species have been reported; however, actual infection rates are unknown or estimated. Some of these species have been identified and are listed in Table 18.2. For a number of these species, the food source is often fish rather than mollusks.

Heterophyes heterophyes

H. heterophyes is one of the smallest trematodes to infect humans and is acquired through the ingestion of pickled or uncooked fish. The heterophyids are capable of parasitizing birds and mammals, and infection was first reported by Bilharz in 1851 (11, 12).

Life Cycle and Morphology

The adult worms measure 1.0 to 1.7 mm in length by 0.3 to 0.4 mm in width, are gray, and have a broadly rounded posterior end (Fig. 18.5 and 18.9). They lay embryonated eggs in the intestinal lumen, and the eggs are discharged into the environment via the feces. The eggs are small, brownish yellow, and operculate, and they possess very subtle opercular shoulders. They measure 27 to 30 μm by 15 to 17 μm (Table 18.1; Fig. 18.4). The eggs are ingested by the snail intermediate host before the miracidium hatches. A sporocyst and one or two redia generations develop in the snail before the production and release of cercariae. The cercariae encyst under the scales or in the flesh of freshwater fish. Humans become infected by eating raw, pickled, or poorly cooked fish containing the metacercariae (10). The metacercariae excyst in the intestinal tract, attach to the walls of the small intestine, and grow to maturity. The adult worm, in addition to an oral and ventral sucker, contains a third sucker, the genital sucker, surrounding the genital pore. The eggs tend to appear in the feces approximately 2 to 3 weeks after infection.

Clinical Disease

Following ingestion of metacercariae, the prepatent period is approximately 9 days. The adult worms cause little damage

TABLE 18.2 Other echinostome infections reported from humans[a]

Trematode	Location	Host	No. and location of spines	Infection acquired
Echinostoma malayanum	Northeastern and northern Thailand	Rats, dogs	43, crown	Freshwater snails, usually cooked
Echinostoma revolutum	Taiwan (3–7% infection rate)	Ducks, geese, rats	37, circumoral	Raw mollusks
Echinostoma lindoense	Indonesia (Lindu Valley; infection may no longer be present in this area); found in Brazil	Rats, mice	37, collar	Raw mollusks
Hypoderaeum conoideum	Northeastern Thailand	Ducks, other fowl, rats, humans	Two rows of 45–53	Salted or raw snails or clams
Echinostoma cinetorchis, *Psilorchis hominis*[b]	Japan, Taiwan			Tadpoles, frogs
Euparyphium melis, *E. beaveri*	Romania, China; may also be in Douglas Lake, Michigan (not studied)		27, crown	Tadpoles
Hypoderaeum cibiuduyn	Northeast Thailand (55% infection rate)	Ducks, geese, fowl		Raw snails
Artyfechinostomum mehrai	India (two cases)	Pig	39–42, collar	Raw snails
Echinostoma recurvatum	Taiwan, Indonesia, Egypt	Rats	45, crown	Tadpoles, frogs
Echinoparyphium paraulum[c]	Former USSR	Ducks, geese, swans, doves		
Himasthla muehlensi	New York City		32, circumoral	Raw clams
Echinochasmus perfoliatus	Hungary, Italy, Romania, former USSR, Far East	Dogs, cats, pigs, foxes	24, circumoral	Raw freshwater fish

[a] Data from reference 3.
[b] May or may not be an echinostome; specimens may have lost crown spines.
[c] May be the same as *Echinostoma revolutum*.

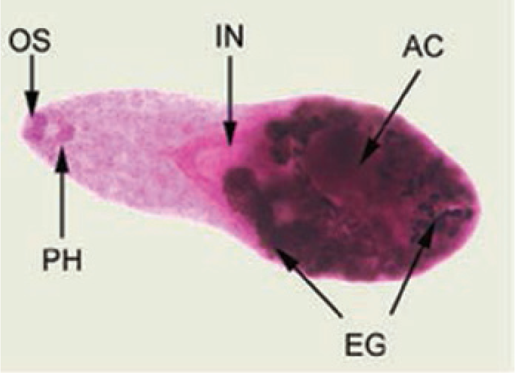


Figure 18.9 *Heterophyes heterophyes* adult worm, stained with carmine. These worms tend to have a broadly rounded posterior end, measure approximately 1 to 1.7 mm in length by 0.3 to 0.4 mm in width, and tend to be gray. In this figure, the following structures are labeled: oral sucker (OS), pharynx (PH), intestine (IN), ventral sucker or acetabulum (AC), and eggs within the uterus (EG). (Courtesy of CDC Public Health Image Library.) doi:10.1128/9781555819002.ch18.f9

to the intestinal tract mucosa except for a mild inflammatory reaction. In heavy infections, the worms cause abdominal pain, mucous diarrhea, and ulceration of the intestinal wall. Because of the small size of the eggs and the fact that the adult may attach itself deeply into the intestinal wall, some of the eggs may end up in the general circulation of the host. The eggs may provoke pathologic lesions, particularly in the heart and brain (13, 14). In the Philippines, 15% of fatal heart disease may be a result of heterophyid myocarditis. Neurologic manifestations due to adult worms or eggs in the brain have been reported (5).

Diagnosis

The diagnosis is suggested by detecting the eggs in the stool. Because the eggs of *H. heterophyes* are similar in shape and size to those of *Metagonimus yokogawai* and *Clonorchis sinensis*, differentiation must be made on the basis of patient history, clinical findings, or recovery of adult worms in the stool.

In Kobe, Japan, *H. katsuradai*, a closely related species that is broader and more rounded, with a very large ventral sucker and smaller eggs (25 to 26 μm by 14 to 15 μm), has been recovered after therapy in patients with diarrhea. Apparently, infection is acquired from the ingestion of raw mullet.

KEY POINTS—LABORATORY DIAGNOSIS

Heterophyes heterophyes

1. The eggs are found in the stool; rarely, adult worms are found in patients with heavy infections after therapy or at autopsy.
2. The eggs of *H. heterophyes*, *M. yokogawai*, and *C. sinensis* appear almost identical and are very difficult to differentiate from one another. When passed, each egg contains a miracidium larva, but the eggs are ingested by the snail before they hatch.
3. The formalin-ethyl acetate sedimentation concentration is recommended for egg recovery; operculated eggs do not float in the zinc sulfate flotation concentration method.
4. The eggs have very inconspicuous opercular shoulders and, unlike *C. sinensis*, lack the "seated" operculum and knob at the abopercular end. *H. heterophyes* eggs are some of the smallest trematode eggs seen and may be missed using the low power of the microscope (10× oculars and 10× objective); high dry power may be required for identification (10× oculars and 40× objective).

Treatment

Praziquantel is the drug of choice and can be given in a single dose of 15 to 25 mg/kg at bedtime.

Epidemiology and Prevention

Numerous fish-eating mammals, including dogs, cats, and birds, act as reservoirs (11). The infection is found in China, Greece, Egypt, India, Iran, Italy, Israel, Japan, Korea, the Philippines, Sudan, Taiwan, Tunisia, and Turkey. Snails serving as the first intermediate host include *Pironella* and *Cerithidea* spp., and a variety of freshwater fish can serve as the second intermediate hosts (15, 16).

Humans become infected by ingestion of parasitized fish that is raw, inadequately cooked, or improperly pickled or salted. Metacercariae can survive for up to 7 days in salted fish. Off the coast of Israel a brackish-water fish, *Mugil capito*, can be heavily infected with *H. heterophyes*, with metacercariae numbering 2,300 to 6,000 per g of fish. The life cycle could be disrupted by improved sanitary conditions and educational information about proper sewage disposal away from ponds or lakes where the intermediate hosts reside.

Metagonimus yokogawai

M. yokogawai was first discovered by Katsurada in 1911 in an infected human (17). The infection is found most often in the Far East and clinically resembles that of *H. heterophyes*. *M. yokogawai* is the most common intestinal fluke infecting humans in the Far East.

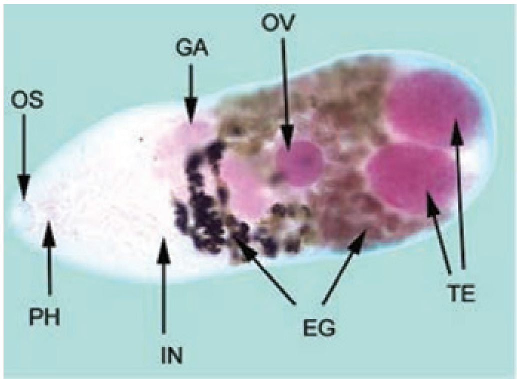


Figure 18.10 *Metagonimus yokogawai* adult worm, stained with carmine. These worms are quite small, like *Heterophyes*, and measure 1 to 2.5 mm by 0.4 to 0.75 mm. The ventral sucker is deflected to the right of the midline and is visible in this image. In this figure, the following structures are labeled: oral sucker (OS), pharynx (PH), intestine (IN), genitoacetabulum (GA), ovary (OV), the large, paired testes (TE), and eggs within the uterus (EG). (Courtesy of CDC Public Health Image Library; image courtesy of Conchology, Inc., Mactan Island, Philippines.) doi:10.1128/9781555819002.ch18.f10

Life Cycle and Morphology

The adult worm measures 1.0 to 2.5 mm by 0.4 to 0.8 mm, attaches to the mucosal wall of the small intestine, and lays embryonated eggs that are passed out with the feces into the environment (Fig. 18.10). The egg is small, brownish yellow, and operculate, with minimal opercular shoulders; it measures 26 to 28 μm by 15 to 17 μm (Table 18.1). The eggs are ingested by the snail intermediate host, *Semisulcospira*, before the miracidium is liberated. On infection of the snail, a sporocyst generation and two redia generations develop before cercariae are liberated. Cercariae encyst under the scales or in the flesh of freshwater or brackish-water fish, which are eaten uncooked or pickled by humans. The metacercariae excyst in the intestinal tract, attach to the mucosal wall, and develop to mature worms. A *Metagonimus* adult is slightly larger than a *Heterophyes* adult and has a ventral sucker that is deflected to the right of the midline axis. The genital pore is attached to the outer rim of the ventral sucker.

Clinical Disease

Symptoms and pathologic changes are similar to those produced by *H. heterophyes* and depend largely on the worm burden of the host. Clinical symptoms are generally mild and transient unless the patient is heavily infected or immunocompromised.These symptoms can include intermittent diarrhea, nausea, and abdominal pain; there may also be eosinophilia in the early stages of infection. Eggs may infiltrate into the intestinal capillaries and lymphatics and be carried to the myocardium, brain, spinal cord, and other tissues, where emboli or granulomatous reactions may occur. In these cases, serious sequelae can occur and include seizures, neurologic deficits, and cardiac insufficiency.

Major histopathological findings include villous atrophy and crypt hyperplasia, with variable degrees of inflammatory reactions. The infected mucosa shows blunting and fusion of the villi, edema of the villus tips, congestion and inflammatory cell infiltrations in the villous stroma, and decreased villus/crypt height ratios.

Diagnosis

The diagnosis is based on recovery of the eggs in the feces. Because the eggs of *M. yokogawai* are similar in shape and size to those of *H. heterophyes* and *C. sinensis*, the definitive diagnosis must be made on the basis of clinical findings, patient history, or recovery of adult worms after therapy or at autopsy. It is documented that *M. yokogawai* adults tend to burrow into the intestinal mucosa, while *H. heterophyes* tends not to invade the intestinal mucosa unless it is a heavy infection. Based on these differences, if a patient presents eggs in the stool *and* demonstrates extraintestinal signs, it is more likely, but not definitive, that the individual is infected with *M. yokogawai*.

KEY POINTS—LABORATORY DIAGNOSIS

Metagonimus yokogawai

1. The eggs are found in the stool; rarely, adult worms are found in patients with heavy infections after therapy or at autopsy.
2. The eggs of *M. yokogawai*, *H. heterophyes*, and *C. sinensis* appear almost identical and are very difficult to differentiate from one another. When passed, each egg contains a miracidium larva, but the eggs are ingested by the snail before they hatch.
3. The formalin-ethyl acetate sedimentation concentration is recommended for egg recovery; operculated eggs do not float in the zinc sulfate flotation concentration method.
4. The eggs have inconspicuous opercular shoulders but a more obvious operculum than *H. heterophyes* eggs and are some of the smallest trematode eggs seen. *M. yokogawai* eggs do not really have the "seated" operculum and knob at the abopercular end like those seen in *C. sinensis* eggs. They may be missed using the low power of the microscope (10× oculars and 10× objective), and high dry power may be required for identification (10× oculars and 40× objective).

Treatment

Praziquantel is the drug of choice and can be given in a single dose of 15 to 25 mg/kg at bedtime, with a 95 to 100% cure rate.

Epidemiology and Prevention

Reservoir hosts are similar to those of *H. heterophyes*. The infection is found in China, Indonesia, Israel, Japan, Korea, Russia, Siberia, Spain, Taiwan, and the Balkans. *M. yokogawai* is considered the most common intestinal fluke infection in the Far East; the prevalence tends to be high in Japan, Korea, and Taiwan, with rates of 2 to 50% being reported from Japan. Freshwater snails (*Semisulcospira* spp.) act as the first intermediate hosts, and a variety of freshwater fish act as the second intermediate hosts.

Preventive measures are similar to those proposed for *H. heterophyes*. Improved education and sanitary standards would greatly reduce the risk of infections.

Other heterophyid species have been found in areas of Asia where people eat raw or poorly cooked freshwater fish. A list of these trematodes can be found in Table 18.3.

Aquaculture. Aquaculture of fish has increased dramatically from 5.3% in 1970 to 32.2% in 2000 (10). Freshwater production has increased greatly and now accounts for 45.1% of the total aquaculture production. The production of grass carp, an important species that serves as an intermediate host for foodborne trematodes, has increased from 10,000 tons in 1950 to 3 million tons in 2002. This growth must be monitored for potential problems related to increased disease in which infection is transmitted through the ingestion of raw or poorly cooked fish. It is estimated that by 2030, at least half of the globally consumed fish will come from aquaculture.

TABLE 18.3 Other heterophyid infections reported in humans[a]

Organism[b]	Comments
Centrocestus formosanus, *Centrocestus* spp.	Reservoir hosts are fish-eating birds and mammals; reported from Taiwan and mainland China
Haplorchis pumilio, *H. yokogawai*, *Haplorchis* spp.	Reservoir hosts are fish-eating birds and mammals; reported from Taiwan, the Philippines, Indonesia, and Thailand
Metagonimus minutus, *Diorchitrema formosanum*, *D. amplicaecale*, *Metagonimus* spp.	Reservoir hosts are fish-eating birds and mammals; reported from Taiwan
Stellantchasmus falcatus, *Stellantchasmus* spp.	Reservoir hosts are fish-eating birds and mammals; reported from Hawaii (from eating raw mullet), Japan, the Philippines, Palestine, Vietnam, Korea, and Thailand
Pygidiopsis summa	Reservoir hosts are fish-eating birds and mammals; reported from Korea (up to 4,000 worms have been recovered from patients)

[a] Intestinal lesions are similar to those produced by *H. heterophyes* and *M. yokogawai*. Eggs of *H. yokogawai*, *H. pumilio*, and *H. taichui* have been found in cardiac lesions but rarely cause complications such as vascular occlusion. Treatment with praziquantel in a single dose of 15 to 25 mg/kg is effective.

[b] From Chai JY et al, *Korean J Parasitol* **47**:S69–S102, 2009.

Freshwater fish aquaculture has increased from an annual production of 136,000 tons in 1952 to 16.6 million tons 50 years later in China, the Republic of Korea, and Vietnam. Most (97.6%, 16.2 million tons) of this fish is produced in China (18). The farmed fish population has increased 100-fold globally in 60 years: from 320,000 tons in 1950, aquaculture fish production reached 32,000,000 tons in 2007.

Suitable conditions for the transmission of foodborne trematodes occur in different ecosystems (waste-fed, traditional, or wild aquaculture systems) and depend on environmental and climatic conditions, the presence of reservoir hosts, and the contamination of water bodies from human defecation and animal feces, among others (19).

Gastrodiscoides hominis

Gastrodiscoides hominis was first recovered in the late 1800s by Lewis and McConnell from an Indian patient suffering from diarrhea (3). The infection is commonly found in Assam, India. Although gastrodiscoidiasis was initially supposed to be restricted to Asian countries, this infection is now being reported in African countries (4). As a common parasite of humans and pigs, it can be found in China, India, Kazakstan, Myanmar, Pakistan, the Philippines, Thailand, Vietnam, and the Volga Delta in Russia, and in Indian immigrants in Guyana.

Life Cycle and Morphology

The complete life cycle is unknown but is probably similar to that of other amphistome trematodes; aquatic plants, crustaceans, and amphibians are thought to be involved. The adult worm is bright pink, can be found attached to the cecum and ascending colon in humans, and may produce a mucous diarrhea. In India, the planorbid snail *Helicorbis coenosus* serves as an intermediate host for this infection.

The eggs of *G. hominis* are passed in feces unfertilized, usually into some kind of water source where they are ingested by the snail intermediate host. The eggs hatch and release miracidia which develop into the sporocyst stage followed by one or more generations of redia. Finally, the redia transform into the cercarial stage. This entire process occurs in approximately 20 days. The cercaria search and penetrate a second intermediate host, such as a fish, where they encyst as metacercaria. These metacercaria can attach to vegetation as another option. The human ingests the metacercaria (approximately 30 to 150 days after being in the snail) either in the infected fish or on contaminated vegetation.

The living worm measures approximately 4 to 5 mm by 8 to 14 mm, is usually bright pink, and is pyriform in outline with a conical anterior (Fig. 18.5 and 18.11). The anterior portion measures about 2 mm. The ventral surface has a prominent acetabulum at the posterior end that bears a characteristic notch. The eggs are greenish brown, operculated, and immature when laid; they measure 60 to 70 µm by 150 µm.

Clinical Disease

The adult worms live in the cecum and ascending colon; symptoms are related to the overall worm burden. In light infections the patient may be asymptomatic, while in heavier infections there may be a mucous diarrhea; lymphocytes, plasma cells, and eosinophils infiltrate the mucosa and submucosa (8). At the attachment site, papular lesions with surface desquamation may lead to necrosis.

Diagnosis

The diagnosis is based on recovery of eggs in the feces. They resemble the eggs of *F. buski* but tend to be more slender. A definitive diagnosis could be made on the basis of clinical findings, patient history, geographic area, or recovery of adult worms after therapy or at autopsy.

KEY POINTS—LABORATORY DIAGNOSIS

Gastrodiscoides hominis

1. The eggs are found in the stool; rarely, adult worms are found after therapy or at autopsy in patients with heavy infections.
2. The eggs of *G. hominis*, *F. buski*, *E. ilocanum*, *F. hepatica*, and *F. gigantica* appear very much alike and can be difficult to differentiate from one another. When passed, these eggs do not contain mature larvae but instead contain undifferentiated embryos. The egg of *G. hominis* tends to be a bit smaller and more slender than that of *F. buski*, but they are very similar.
3. The formalin-ethyl acetate sedimentation concentration is recommended for egg recovery; operculated eggs do not float in the zinc sulfate flotation concentration method.
4. The less mature the egg, the more difficult it may be to see the actual operculum—it blends into the shell outline, and the "breaks" in the shell may be difficult to identify. This egg has no opercular shoulders, and so it is difficult to see where the operculum "breaks" in the shell actually occur.

Treatment

Praziquantel is the drug of choice and is administered as a single 15-mg/kg dose after the evening meal or at bedtime. A second regimen consists of 75 mg/kg/day in three doses for 1 day. Apparently, children tend to tolerate the drug better than adults.

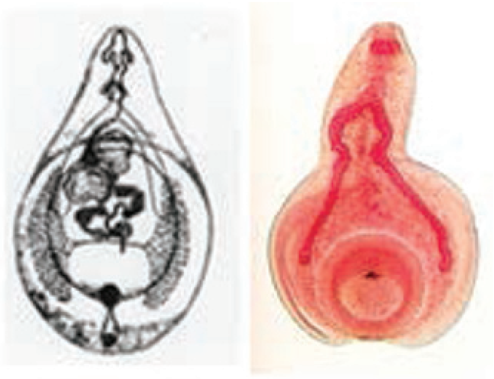


Figure 18.11 *Gastrodiscoides hominis* adult worms. Note the pyriform shape and the deep concavity on the ventral surface, which contains a large sucker. The adult worms measure 8 to 14 mm in length by 4 to 5 mm in width and are usually bright pink. (Illustration by Sharon Belkin. Adapted from references 3 and 8.) (Right image courtesy of http://www.med.cmu.ac.th/dept/parasite/trematodes/GhAd.htm.)
doi:10.1128/9781555819002.ch18.f11

Epidemiology and Prevention

G. hominis is commonly found in Asia and has been reported from Assan, Bihar, and Orissa in India, as well as from China, Kazakhstan, Myanmar, the Philippines, Thailand, Vietnam, and Guyana. One study in India found a 41% prevalence rate in which pigs were the reservoir host. Other reservoir hosts include deer mice in Malaysia and rats in Indonesia, Japan, and Thailand. Prevention of this disease is not difficult when simple sanitary measures are taken. Night soil should never be used as a fertilizer because it could contain any number of parasites, including the eggs of *G. hominis* in endemic areas. All fish should be thoroughly cooked and/or potentially contaminated vegetation should be thoroughly washed before ingestion.

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19

Liver and Lung Trematodes

Liver Flukes

Clonorchis sinensis

Opisthorchis viverrini

Opisthorchis felineus

Fasciola hepatica

Fasciola gigantica

Less Common Liver Flukes

Dicrocoelium dendriticum, *Dicrocoelium hospes*, and *Eurytrema pancreaticum*

Lung Flukes

Paragonimus westermani

Paragonimus kellicotti

Clonorchis, *Opisthorchis*, and *Fasciola* spp. are trematodes that parasitize the biliary ducts of humans. *Clonorchis* and *Opisthorchis* spp. are narrow, elongate worms that localize in the more distal, smaller ducts of the biliary tree. *Fasciola hepatica*, because of its much larger size, resides in the larger bile ducts and gallbladder.

Paragonimus spp., also known as lung flukes, cause paragonimiasis in humans. The adults encapsulate in the lungs and are occasionally found in other body sites.

The infections caused by the liver and lung trematodes are food borne and have considerable economic and public health impact. It is estimated that more than 50 million people have acquired foodborne trematode infections. It is estimated that 601 million, 293 million, 91 million, and 80 million people are at risk of infection with *Clonorchis sinensis*, *Paragonimus* spp., *Fasciola* spp., and *Opisthorchis* spp., respectively (1). Of great public health concern is cholangiocarcinoma associated with *Clonorchis* and *Opisthorchis* infections, severe liver disease associated with *Fasciola* infections, and the misdiagnosis of tuberculosis in those infected with *Paragonimus* spp.

Liver Flukes

Clonorchis sinensis

McConnell was the first to describe the adult worms and pathologic changes caused by *C. sinensis*, in a Chinese patient who died in Calcutta, India. The complete life cycle was developed in a number of publications by Iijima in 1887, Saito in 1898, Kobayashi in 1914, and Muto in 1918 (2). *C. sinensis* is also known as the Chinese or oriental liver fluke (Table 19.1).

Life Cycle and Morphology

The definitive hosts of *C. sinensis* are humans, dogs, hogs, cats, martens, badgers, mink, weasels, and rats. Adult worms deposit eggs in the bile ducts, and the eggs are discharged with the bile fluid into the feces and passed out into the environment (Fig. 19.1). An adult fluke can produce 1,000 to 4,000 eggs/day for at least 6 months. The eggs are fully embryonated when laid and measure 28 to 35 µm by 12 to 19 µm (Fig. 19.2). The eggs are ovoid, with a thick, pale

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TABLE 19.1 Characteristics of liver and lung trematodes

Species	Distribution	Agent of infection	Reservoir host	Egg		
				Size (µm)	Morphology	Comments
Clonorchis sinensis	Far East	Uncooked fish	Dogs, cats, other fish-eating mammals	28–35 by 12–19	Embryonated, operculated	Very prominent opercular shoulders; has a "seated" operculum and knob at the abopercular end.
Opisthorchis viverrini	Northern Thailand, Laos	Uncooked fish	Dogs, cats, other fish-eating mammals	19–29 by 12–17	Embryonated, operculated	Prominent opercular shoulders; has a "seated" operculum and may or may not have a knob at the abopercular end; eggs tend to be broader with less prominent shoulders than *C. sinensis* eggs.
Opisthorchis felineus	Poland, Germany, Russian Federation, Kazakhstan, western Siberia	Uncooked fish	Dogs, cats, other fish-eating mammals	28–30 by 11–16	Embryonated, operculated	Prominent opercular shoulders; has a "seated" operculum and may or may not have a knob at the abopercular end; eggs tend to be broader with less prominent shoulders than *C. sinensis* eggs.
Fasciola hepatica	Worldwide; mixed *F. hepatica* and *F. gigantica* infections have been reported from Pakistan	Uncooked water plants	Herbivores	130–150 by 63–90	Unembryonated, operculated	The less mature the egg, the more difficult it may be to see the actual operculum—it blends into the shell outline, and the "breaks" in the shell may be difficult to identify. This egg has no opercular shoulders, and so it is difficult to see where the operculum breaks in the shell actually occur. Eggs resemble those of *F. buski*, *E. ilocanum*, *F. gigantica*, and *G. hominis*; eggs may have thickening at the abopercular end of the shell (unlike eggs of *F. buski*).
Fasciola gigantica	Africa, southern Europe, southern United States, Hawaii, former Soviet Union, Middle East, Southeast Asia	Uncooked water plants	Herbivores	160–190 by 70–90; egg is larger than *F. hepatica*	Unembryonated, operculated	The egg is larger than that of *F. hepatica*; both *F. hepatica* and *F. gigantica* worms have been found in ectopic sites.
Dicrocoelium dendriticum, *D. hospes*, *Eurytrema pancreaticum*	Europe, Turkey, northern Africa, Far East, China, Japan, North and South America	Ants, grasshoppers, crickets	Cattle, sheep, deer, water buffalo	38–45 by 22–30; dark brown	Embryonated, operculated, thick shell	Eggs have a thick, dark brown shell and essentially no opercular shoulders; they cannot be differentiated from each other.
Paragonimus westermani	Far East, Africa	Crabs, crayfish	Dogs, cats, tigers, lions	80–120 by 45–65	Unembryonated, operculated	Eggs have a moderately thick, dark golden-brown shell, a prominent operculum, opercular shoulders, and a thickened abopercular end; may be confused with *D. latum* eggs (smaller, abopercular knob).
P. kellicotti	North America	Crayfish	Dogs, cats, bobcats, raccoons, foxes, skunks, minks, coyotes	75–118 by 48–68	Unembryonated, operculated	Eggs have a moderately thick, dark golden-brown shell, a prominent operculum, opercular shoulders, and a slightly thickened abopercular end; tapers more sharply than *P. westermani*; may be confused with *D. latum* eggs (smaller, abopercular knob).
P. mexicanus	Central and South America	Crabs	Opossum, cats, dogs	60–70 by 42–56	Unembryonated, operculated	Thin, irregular undulations on outer shell; eggs have a prominent operculum, opercular shoulders, and a thickened abopercular end; may be confused with *D. latum* eggs (smaller, abopercular knob); eggs smaller than *P. westermani;* also golden-brown shell.

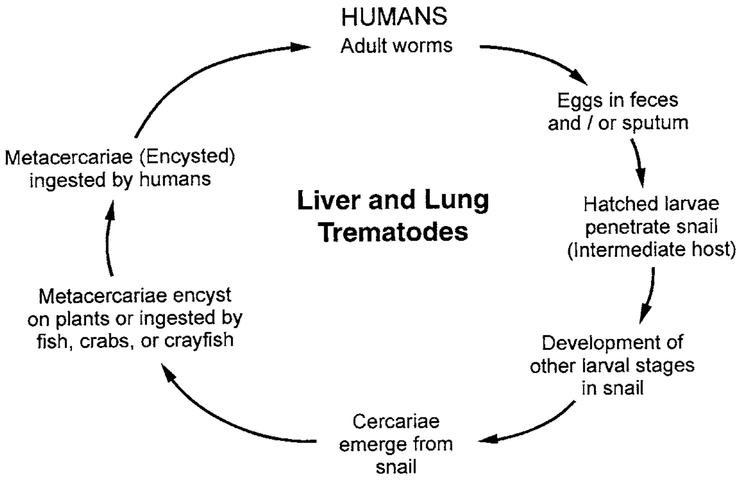

HUMANS
Adult worms
Eggs in feces and / or sputum
Hatched larvae penetrate snail (Intermediate host)
Development of other larval stages in snail
Cercariae emerge from snail
Metacercariae encyst on plants or ingested by fish, crabs, or crayfish
Metacercariae (Encysted) ingested by humans
Liver and Lung Trematodes

Figure 19.1 Life cycle of the liver and lung trematodes. doi:10.1128/9781555819002.ch19.f1

brownish yellow shell and an operculum. There are distinct opercular shoulders surrounding the operculum. Frequently, the eggs contain a comma-shaped appendage at the abopercular end. Eggs are ingested by the snail host, at which time the miracidium hatches to infect the snail; *Parafossarulus* and *Bithynia* are the most suitable hosts. More than 100 species of snails can serve as an intermediate host for *C. sinensis*. The snails generally live in ponds used for commercial fish raising, lakes, and slow-moving water, and the overall infection rate is relatively low (3). Sporocyst and redia generations are produced in about 4 to 5 weeks before cercariae are released to encyst in the skin or flesh of freshwater fish. Numerous species of freshwater fish, mostly belonging to the family Cyprinidae, serve as the second intermediate host. After a few days in the fish muscle, the cercariae become encysted and form metacercariae. Humans become infected by ingesting the metacercariae in raw or incompletely cooked fish.

Metacercariae excyst in the duodenum, enter the common bile duct, and travel to the distal bile capillaries, where the worms mature over a period of about a month (Fig. 19.3 and 19.4). The life cycle takes approximately 3 months to complete in humans. Reservoir hosts include dogs, cats, pigs, mink, rats, and other fish-eating mammals.

The adult worm is a small trematode with an elliptical shape and an average length of 10 to 25 mm. The trematode is a true hermaphrodite and has a life span of 20 to 25 years, which explains the persistent infection for a long duration.

Clinical Disease

Most patients with mild infections of fewer than 100 flukes have few symptoms, which may include general malaise, abdominal discomfort, and diarrhea. In 10 to 40% of patients, peripheral eosinophilia occurs with fluctuating obstructive jaundice. Moderate infections with fewer than 1,000 worms include symptoms of fever and chills, fatigue, anorexia, diarrhea, weight loss, discomfort, and abdominal distention. In severe disease with up to 20,000 flukes, an additional symptom may include acute right upper quadrant pain. In the late stage of severe cases, jaundice, diarrhea, portal hypertension, hepatosplenomegaly, ascites, and edema can occur.

In general, the complications of clonorchiasis are the result of biliary obstruction. As the worms mature in the

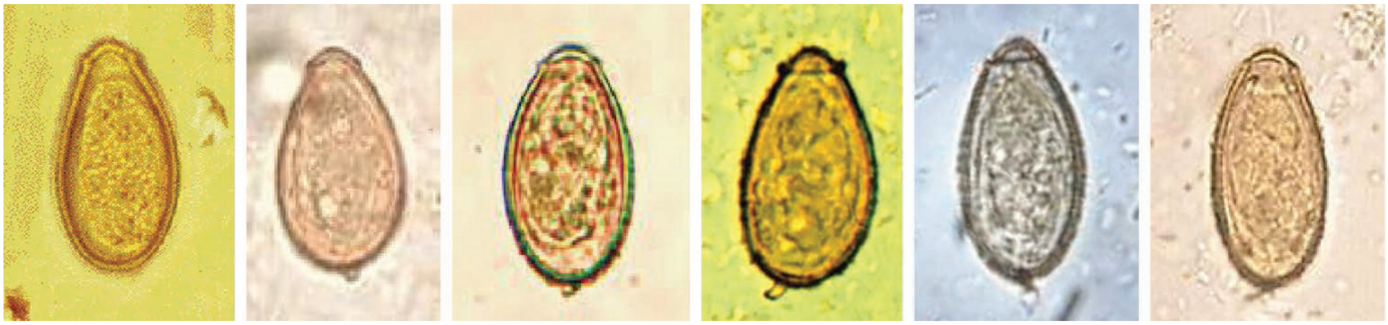


Figure 19.2 Eggs of *Clonorchis sinensis*. Note the range of sizes and shapes. doi:10.1128/9781555819002.ch19.f2

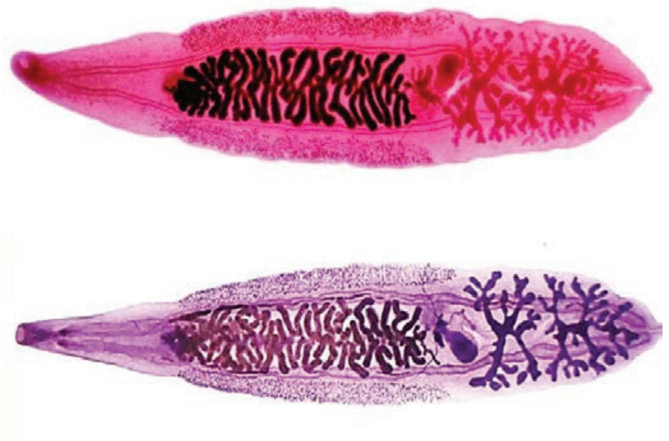
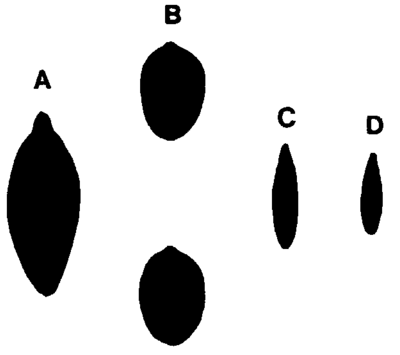


Figure 19.3 *Clonorchis sinensis* adults (10 to 25 mm long by 3 to 5 mm wide). (Upper, Armed Forces Institute of Pathology photograph; lower, CDC Public Health Image Library.) doi:10.1128/9781555819002.ch19.f3

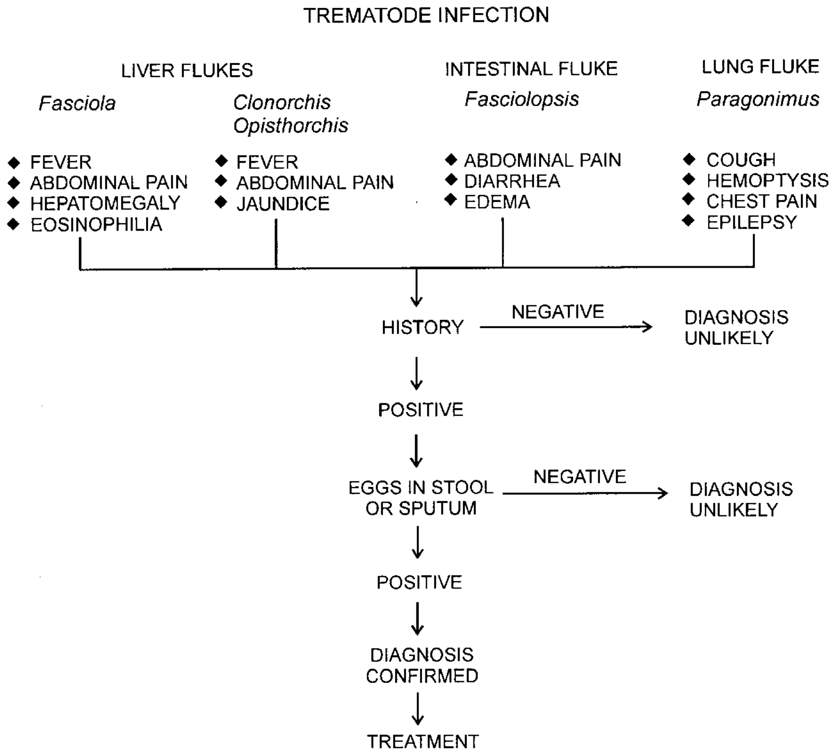
distal bile ducts, an inflammatory response is seen in the biliary epithelium. The extent of pathologic changes is related to the intensity and duration of infection (Algorithm 19.1). The lesions are confined mainly to the biliary system and are the result of mechanical irritation and toxins produced by the worms. In light infections, there appears to be little or no change in liver parenchyma, whereas heavy infections cause thickening and localized dilations of the bile ducts with hyperplasia of the mucinous glands. As a result, the biliary tract may become obstructed, causing bile retention, infiltration of lymphocytes and eosinophils, and fibrosis (Fig. 19.5). The adenomatous changes may persist for many years in patients with light infections. The infections have been associated with obstructive jaundice, which may be aggravated by biliary stones and liver abscesses. Many patients infected with *C. sinensis* have recurrent pyogenic cholangitis. There is no direct evidence that infection with *C. sinensis* causes chronic bacterial infection. Acute pancreatitis, cholecystitis, and cholelithiasis may be the result of worm invasion. Cirrhosis is probably related to malnutrition rather than parasitic infections. Evaluation by computed tomography (CT) has shown that in the presence of diffuse mild intrahepatic bile duct dilation, enlargement of the body or tail (or both) of the pancreas, with a cluster of small cystic changes within the pancreatic parenchyma, provides strong evidence of *C. sinensis* pancreatitis (4).

Figure 19.4 Adult liver and lung trematodes. (A) *Fasciola hepatica*; (B) *Paragonimus westermani*; (C) *Clonorchis sinensis*; (D) *Opisthorchis viverrini* and *O. felineus*. doi:10.1128/9781555819002.ch19.f4



In light infections, the patient generally experiences no symptoms. In heavier infections acquired over time, the patient may experience dull pain and abdominal discomfort that may last for 1 to 2 h, often in the afternoon. As the disease progresses, the duration of pain lengthens and the pain may become so severe that the patient is unable to work. Patients who have had the disease for a long time show liver enlargement with some degree of functional impairment that is secondary to biliary obstruction. Acute infections caused by ingestion of large numbers of metacercariae cause fever, chills, diarrhea, epigastric pain, enlarged tender liver, and possibly jaundice within a month of ingestion. The acute symptoms last for about 1 month and subside at about the time when eggs are detected in the stool. In chronic infections, cholangitis, cholelithiasis, pancreatitis, and cholangiocarcinoma are common complications and can lead to death (5, 6).

Cholangiocarcinoma. Liver flukes play a major role in East Asia, while exposure to chemical compounds, such as naphthenic acids, has been postulated as a source of the rate increase of cholangiocarcinoma in Western countries. *C. sinensis* has been linked to neoplasms of the bile duct and to cholangiocarcinoma (5, 7), which is most frequently observed in areas where clonorchiasis is endemic. Cholangiocarcinoma is a malignant tumor that arises from the bile duct epithelium and is the second most prevalent liver cancer after hepatocellular carcinoma. The tumor usually occurs in patients 60 to 80 years of age and rarely in patients younger than 40 years. There appears to be no direct link between infection and carcinoma, although one of the first steps in malignant transformation may be induced by the biliary tract hyperplasia caused by the worms. Patients with primary sclerosing cholangitis (PSC) have a substantial predisposition for bile duct carcinoma. Although the exact mechanisms are not well defined, longstanding inflammation can lead to cholangiocarcinoma in patients with chronic *C. sinensis* infection. PSC is an uncommon disease, characterized by stricturing, fibrosis, and inflammation of the biliary tree, which is closely associated with chronic inflammatory bowel disease, particularly ulcerative colitis. The only effective treatment for PSC is


TREMATODE INFECTION
LIVER FLUKES
Fasciola
Clonorchis
Opisthorchis
INTESTINAL FLUKE
Fasciolopsis
LUNG FLUKE
Paragonimus
◆ FEVER
◆ ABDOMINAL PAIN
◆ HEPATOMEGALY
◆ EOSINOPHILIA
◆ FEVER
◆ ABDOMINAL PAIN
◆ JAUNDICE
◆ ABDOMINAL PAIN
◆ DIARRHEA
◆ EDEMA
◆ COUGH
◆ HEMOPTYSIS
◆ CHEST PAIN
◆ EPILEPSY
HISTORY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
EGGS IN STOOL OR SPUTUM
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
DIAGNOSIS CONFIRMED
TREATMENT

Algorithm 19.1 Trematode infection.
doi:10.1128/9781555819002.ch19.Alg19.1

orthotopic liver transplantation, which in the absence of cholangiocarcinoma has a 5-year survival rate of 89%. However, patients with cholangiocarcinoma who undergo liver transplantation have a high risk of recurrence and poor survival statistics. Between 10 and 20% of patients with PSC will go on to develop a cholangiocarcinoma (9). Therefore, identification of patients with deteriorating liver function prior to the development of cholangiocarcinoma remains an important goal in the management of PSC, particularly in areas where *C. sinensis* infection is endemic (10). It is also probable that cofactors play a role; liver flukes are promoters and not initiators of cholangiocarcinoma (7). It has been suggested that regular biliary cytologic sampling to detect dysplasia can predict the development of cholangiocarcinoma; however, this approach is unlikely to be widely used. In the Far East, forms of chronic inflammation associated with cholangiocarcinoma include infestation with either of the liver flukes, *C. sinensis* or *Opisthorchis viverrini*.

The interaction of interleukin-6 (IL-6), transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), and platelet-derived growth factor (PDGF) are at the basis of the proliferation of biliary epithelial cells or cholangiocytes. Additional steps for the final development of cholangiocarcinoma probably also involve an increase of the mutation rate of tumor suppressor genes, such as TP53, and the evasion of apoptosis (11).

Diagnosis

Fecal Examination. Individuals who have lived in areas where infections are endemic, have a history of eating raw freshwater fish, and have symptoms of upper abdominal pain, indigestion, diarrhea, and hepatomegaly may be suspected of having liver trematode infection. Eggs can be detected in the feces by direct microscopy or by examination of fecal concentrates. Sedimentation concentration is recommended since these eggs have opercula and do not float in the zinc sulfate flotation concentration method. Sedimented material can be examined using saline or D'Antoni's iodine or both. It is important not to add too much iodine; if the eggs stain too darkly, they may resemble debris. In light infections, egg counts may be low and so multiple specimens may be required to confirm their presence. The eggs of *C. sinensis* (Fig. 19.2) and *O. viverrini* are similar in size and shape to those of *Heterophyes heterophyes* and *Metagonimus yokogawai* and cannot be readily differentiated from them. If a patient has not resided in or recently visited areas where infections are endemic, the infection is probably due to *C. sinensis* or *O. viverrini*. The infection may be confirmed by detecting eggs in the bile fluid (duodenal aspirate), by recovering adult worms, or from the clinical history. Some strains of *C. sinensis* produce eggs that have a comma-shaped appendage at the abopercular end. Multiple egg measurements are usually required to determine size differences; however,

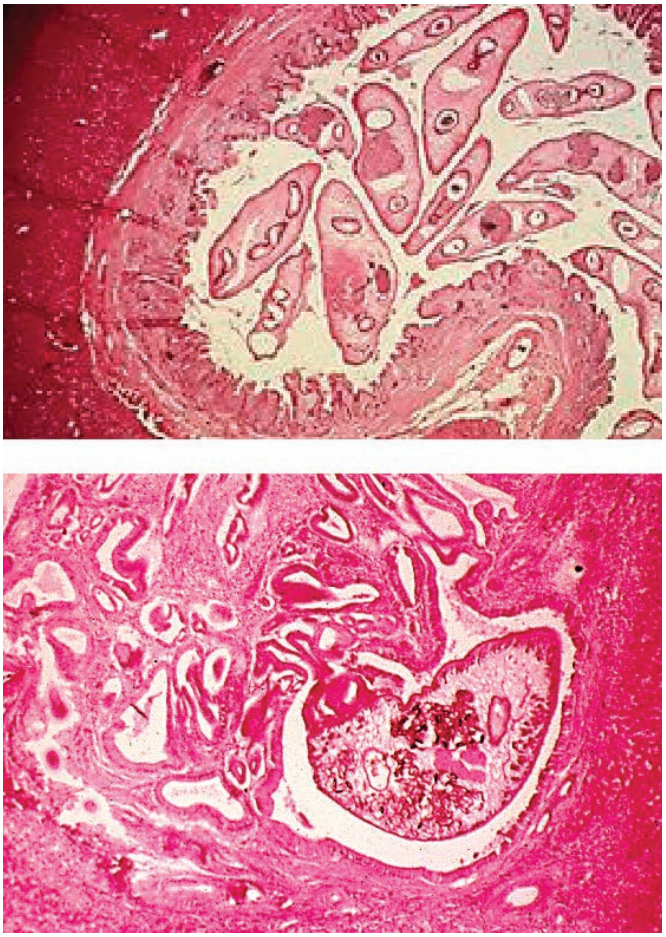


Figure 19.5 *Clonorchis sinensis* adult in a biliary duct (cross section). (Upper, http://usmlepathslides.tumblr.com/post/21383019000/common-bile-duct-with-clonorchis; lower, from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch19.f5

absolute identification among the small trematode eggs can be very difficult. Eggs can also be seen in duodenal drainage material or, if the Entero-Test capsule is used, in the mucus removed from the string. Definitive identification usually requires examination of adult worms recovered after therapy or during surgery or autopsy.

Eggs are generally not found in stool specimens from patients with biliary obstruction. Consequently, needle aspiration, surgery, or autopsy specimens may be required to confirm their presence. In these patients, biliary obstruction must be differentiated from enlarged gallbladder, cholangitis with jaundice, liver carcinoma, and cholangiocarcinoma.

Serologic Methods. IgE and *C. sinensis*-specific IgE levels in serum are elevated in infected individuals. In patients with acute infections, there is an increase in IgM levels in serum followed by increases in IgA and IgG levels. In patients with chronic infections, the IgA level returns to normal while the IgG and IgM levels remain elevated. The intradermal test is no longer used due to its very low specificity. An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *C. sinensis* has also been used (12). A recombinant propeptide of cathepsin L proteinase (rCsCatL-propeptide) used in an ELISA appears to be a valuable candidate for specific IgG4 detection in sera from clonorchiasis patients. This ELISA has minimal cross-reactions and acceptable sensitivity. In addition, specific IgG4 detection can be used to evaluate the level of infection and therapeutic effect in clonorchiasis patients (13). A multiplex PCR approach has proven to be species specific, sensitive, and rapid in the accurate diagnosis of clonorchiasis and/or opisthorchiasis. This test can be used for the detection of metacercariae in infected fish or adult worms or eggs from patients in areas of endemic infection (14).

Radiological Screening. Cholangiography, ultrasonography, and liver scans may reveal lesions consistent with liver fluke infection. Clonorchiasis is often diagnosed incidentally during abdominal ultrasonography, since symptoms tend to be nonspecific. However, none of these methods is any more sensitive than fecal examination because of their limited sensitivity and specificity (15). According to a study carried out in an endemic area, the sensitivity of ultrasonography was 52% and its specificity was 51%. The low sensitivity was due to false-negative cases with mild infection, and the low specificity was due to false-positive cases with residual pathology after cure (16). This low specificity becomes more of an issue as the number of patients cured continues to increase due to extended control measures and changes in the ecological environment. Thus, ultrasonography becomes less helpful in the differentiation between cured and active infections. The pathological changes in the bile ducts may persist for years after cure and may not indicate the presence of the viable worms. CT results are essentially the same as those observed by ultrasonography, with mild, uniform dilatation of the peripheral intrahepatic bile ducts without a focal obstructing lesion.

Peripheral cholangiocarcinoma is usually seen as a large, well-defined hepatic mass with lobulated margins. CT and magnetic resonance imaging (MRI) reveal band-like peripheral contrast enhancement of variable thickness around the tumor during the early phase, with progressive filling of contrast material in the later stages. This appearance occurs through slow diffusion of contrast materials into the interstitial spaces of the tumor. Fibrosis within this type of cholangiocarcinoma also contributes to the delayed contrast enhancement of the tumor. Additional findings in peripheral cholangiocarcinoma include capsular retraction and dilatation of the peripheral intrahepatic ducts (17).

Most hilar and extrahepatic cholangiocarcinomas cause a focal stricture of the bile duct, whereas papillary carcinoma is occasionally found in the form of a polypoid mass within the duct. By thin-section CT, this tumor type can be seen as an ill-defined focal wall thickening. Using ultrasonography, the tumor can appear as a thickening or encircling mass along the bile duct wall. In a number of cases, the infiltrating tumor is seen by either ultrasonography or CT as a simple nonunion of the dilated right and left hepatic ducts without any identifiable mass. Cholangiography shows a focal or diffuse stricture or complete obstruction of the bile ducts; from this image the extent of the disease can be determined (17).

Radiological findings are regarded as pathognomonic for clonorchiasis because they demonstrate the unique pathological changes of this disease. Due to remarkable advances in imaging technology, individual flukes can now be seen using noninvasive radiological techniques.

KEY POINTS—LABORATORY DIAGNOSIS

Clonorchis sinensis

1. Multiple stool specimens may be required to find the eggs.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the small eggs will stain very darkly and resemble debris.
4. Although the wet preparation can be examined using the 10× (low-power) objective, these eggs measure approximately 30 μm and can be easily missed. It is recommended that the 40× (high dry) objective be used for the wet preparation examination.
5. Eggs of *C. sinensis* can resemble those of many other intestinal and liver trematodes.
6. The wet preparation should not be so thick that the small eggs are obscured by normal stool debris.
7. Some strains of *C. sinensis* and *O. viverrini* produce eggs that have a comma-shaped appendage at the abopercular end; this finding generally differentiates the liver fluke eggs from those of the intestinal flukes *Heterophyes heterophyes* and *Metagonimus yokogawai*.
8. If duodenal sampling is performed (drainage or the Entero-Test), eggs may be found in this material.
9. If available, antigen or antibody detection may be helpful in confirming the infection.

Treatment

Praziquantel is the drug of choice for treatment of *C. sinensis* infections. It has very few or limited side effects but is not recommended for use during pregnancy. The recommended dose is 75 mg/kg after meals three times a day for 1 or 2 days (18, 19). These regimens yielded 85 and 100% cure rates, respectively. The majority of patients do well, with the exception of those who have pyogenic cholangitis, obstructive jaundice, or cholangiocarcinoma. Common reactions are usually mild and include abdominal pain, diarrhea, dizziness, sleepiness, and headache. It is recommended that praziquantel be given before sleep at night. However, in rare instances, praziquantel may cause allergic, anaphylactic, or hypersensitivity reactions in some individuals. Albendazole may be used as an alternative drug. Once cholangiocarcinoma is diagnosed, complete resection with negative histologic margins provides the greatest chance for long-term survival. Unfortunately, cholangiocarcinoma responds poorly to chemotherapy.

Epidemiology and Prevention

Humans, dogs, cats, and other fish-eating mammals are reservoir hosts and, in some areas, the sole cause of continual transmission. Recent estimates of the number of cases of this infection in parts of Asia were as follows: China (12.5 million), Hong Kong (333,000), Japan (only occasional cases), Korea (1.17 million), Macao (21,000), Taiwan (89,000), and Vietnam (1 million), for a total of approximately 15 million (3, 20). Infections have also been documented in Russia, Malaysia, other parts of Asia, Europe, North America, South America, Australia, and Africa, but it is no longer endemic in Japan. Natives of Hawaii also harbor the infection, which is contracted through the ingestion of frozen, pickled, or dried fish imported from areas of endemicity. Human infections result from the widely prevalent practice of consumption of metacercaria-infested freshwater fish eaten uncooked, pickled, smoked, salted, or dried. The traditional eating habits which are a part of a cultural heritage are very difficult to change. In the past few years, clonorchiasis has often been found in refugee immigrants from areas of endemic infection who are resident in the United States, Canada, France, or Australia. Participants in laboratory proficiency testing programs have improved their ability to identify these eggs in challenge stool samples, thus increasing the probability that patients who are infected with *C. sinensis* will be diagnosed. In 2009, it was also estimated that about 681 million people worldwide are at risk of infection and more than 46 million people are infected with liver flukes (*C. sinensis* and *Opisthorchis* sp.) (21).

The life cycle is perpetuated by humans or reservoir hosts defecating in or near freshwater sources containing suitable species of snails. In areas where fish cultivation is of economic importance, toilets have been built over ponds to provide a source of fish food. Operculate snails noted to be infected are *Parafossarulus*, *Bulinus*, *Semisulcospira*, *Alocinma*, and *Melanoides* spp. Metacercariae can encyst in numerous species of freshwater fish.

Aquaculture. Fish aquaculture as a proportion of global fish production has increased dramatically from 5.3% in 1970 to 32.2% in 2000 (1). Freshwater fish production has increased dramatically and now accounts for 45.1% of the total aquaculture production. The production of grass carp, an important species that serves as an intermediate host for food-borne trematodes, has increased from >10,000 tons in 1950 to >3 million tons in 2002. Growth of this industry must be monitored for potential problems related to an increased incidence of disease in which infection is transmitted through the ingestion of raw or poorly cooked fish. Freshwater fish aquaculture has increased from an annual production of 136,000 tons in 1952 to 16.6 million tons 50 years later in China, the Republic of Korea, and Vietnam. Most (97.6%, 16.2 million tons) of this fish is produced in China (1). The farmed fish population has increased 100-fold globally in 60 years: from 320,000 tons in 1950, aquaculture fish production reached 32,000,000 tons in 2007. It is estimated that by 2030, at least half of the fish consumed globally will come from aquaculture farming.

In one study of fish farms in Vietnam, the prevalence of fish-borne zoonotic trematodes in fish increased by 11 percentage points during a 1-year culture period. It was also emphasized that the young fish were already highly infected when stocked into the grow-out systems (22).

Prevention. The life cycle can be broken and infection can be prevented in humans by thorough cooking of all freshwater fish. Although cultural habits are difficult to change, public health education can be used to modify them. Night soil used for fertilizer without disinfection should not be applied in lakes or ponds containing susceptible snails. It has been suggested that night soil should be stored prior to use; eggs of *C. sinensis* die within 2 days when stored at 26°C.

Opisthorchis viverrini

Liver fluke infections with *Opisthorchis viverrini*, *Opisthorchis felineus*, and *Clonorchis sinensis* continue to be a major public health problem in many areas within Asia and Eastern Europe. *O. viverrini* has been reported to infect a significant portion of the population in northern Thailand and Laos. In some areas, the prevalence was 100% for age groups older than 10 years, and it is estimated that 10 million people are infected (23) (Table 19.1). Although the overall prevalence rate has been reported to be 35%, there are some areas where it is greater than 90%. There is an association between infection with *O. viverrini* and carcinoma of the bile duct epithelium (cholangiocarcinoma) (24, 25).

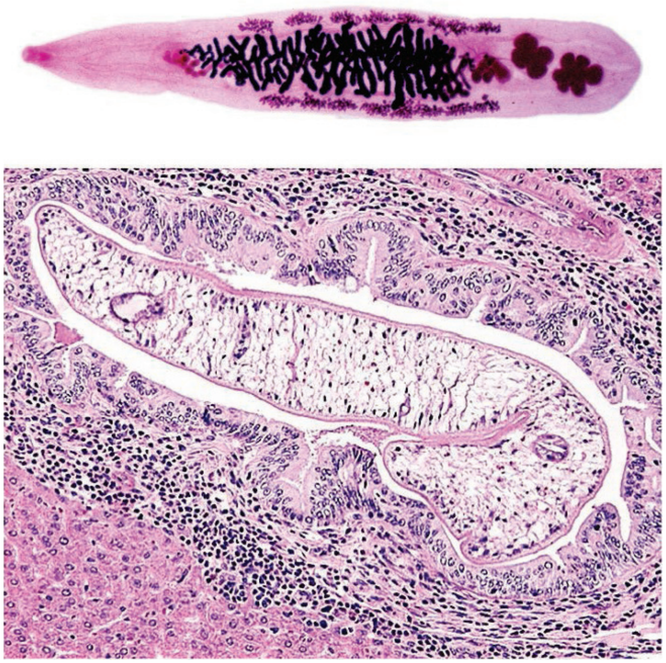
Life Cycle and Morphology

Adult worms in distal bile ducts produce eggs that are carried by the bile to the intestinal lumen and to the outside environment in the feces (Fig. 19.1, 19.4, and 19.6). The eggs are brownish yellow, oval, and operculated; they have opercular shoulders and may have a comma-shaped appendage at the abopercular end. The egg size averages 27 by 15 μm. The life cycle is similar to that of *C. sinensis*, requiring developmental periods in susceptible snails and finally in freshwater fish, in which the metacercariae encyst. Metacercariae excyst in the duodenum of the mammalian host and migrate to the intrahepatic bile ducts, where they develop to mature adults. The adult worms are transparent and leaf-shaped and measure approximately 8 to 12 mm long (Fig. 19.4). The length-to-width ratio is similar to that of *C. sinensis*; therefore, the two can be confused. The life span of *O. viverrini* can exceed 20 years.

Clinical Disease

Individuals who have lived in areas where infections are endemic, who have a history of eating raw fish, and have

Figure 19.6 *Opisthorchis viverrini*. (Upper) Adult trematode (courtesy of CDC Public Health Image Library). (Lower) Adult trematode in bile duct of experimentally infected hamster (from Sripa B, *PLoS Negl Trop Dis* **2**:e232, 2008, doi:10.1371/journal.pntd.0000232). doi:10.1128/9781555819002.ch19.f6



symptoms of right upper abdominal pain, indigestion, diarrhea, and hepatomegaly may be suspected of having liver trematode infection. Pain usually appears in the late afternoon and lasts several hours. This infection is associated with a number of hepatobiliary abnormalities, including cholangitis, obstructive jaundice, hepatomegaly, periductal fibrosis, cholecystitis, and cholelithiasis. As with *C. sinensis*, the pathologic changes are confined mainly to the biliary tract system and morbidity is significantly associated with the worm burden of the host. Intensity of infection is correlated with clinical signs of abdominal pain, flatulence, weakness, hepatomegaly, cholangitis, chronic cholecystitis, cholelithiasis, and obstructive jaundice. Patients with heavy worm burdens may have severe cirrhosis, ascites, pedal edema, and acute abdominal pain. There may be severe jaundice, secondary infection of the biliary system (as seen with *C. sinensis*), cholangitis, and hepatomegaly. The bilirubin transaminase level tends to be elevated, while the serum albumin is low.

Cholangiocarcinoma. The association between cholangiocarcinoma and liver flukes has been documented for approximately 60 years. Apparently, chronic *O. viverrini* infection and the development of cholangiocarcinoma is the strongest association between a parasitic infection and cancer and is even stronger than the link between *Schistosoma haematobium* infection and squamous cell carcinoma. A large number of individuals with *O. viverrini* infection have cholangiocarcinomas, which were confirmed at autopsy. The biliary epithelium may be highly susceptible to malignant transformation because of chronic proliferation due to *O. viverrini* infection. There is a significant relationship between the intensity of infection and cholangiocarcinoma, and there is a high prevalence of the disease among males (7, 24, 25). There is a definite relationship between a high risk of liver cancer and infestation with *O. viverrini* in northeastern Thailand. However, in other areas of the country where the prevalence of liver cancer is high, there is little to no exposure to *O. viverrini* infection. Case-control studies suggest that exposure to exogenous and possibly endogenous nitrosamines in food, tobacco, and betel nut may play a role in the development of hepatocellular carcinoma, while infestation with *O. viverrini* and chemical interaction of nitrosamines may also be etiologic factors in the development of cholangiocarcinoma (26). Carcinomas of the biliary tract are rare cancers developing from the epithelial or blast-like cells lining the bile ducts. A variety of known predisposing factors, including infection with *O. viverrini*, have been identified. Chronic inflammatory processes, generation of active oxygen radicals, altered cellular detoxification mechanisms, activation of oncogenes, functional loss of tumor suppressor genes, and dysregulation of cell proliferation and apoptotic mechanisms have been identified as important contributors to the development of cholangiocarcinomas (25, 27). Approximately 20,000 worms have been recovered from a single individual at autopsy.

As the flukes feed on the biliary epithelium, they cause damage from mechanical injury and the inflammatory response to this process. Excretory-secretory proteins are released from the tegument and excretory pore of the worms into the bile, some of which are highly immunogenic. These proteins have been associated with cancers; they include proteases of different mechanistic classes and are similar to mammalian growth factors and antiapoptotic proteins. A cysteine protease inhibitor has also been identified which, in other helminth pathogens, induces nitric oxide production by macrophages and may contribute to malignant transformation of inflamed cells (28).

Diagnosis

Fecal Examination. Eggs can be detected in the feces by direct microscopy or by examination of fecal concentrates. Sedimentation concentration is recommended since these eggs have opercula and do not float in the zinc sulfate flotation concentration method. Sedimented material can be examined using saline or D'Antoni's iodine or both. It is important not to add too much iodine; if the eggs stain too darkly, they may resemble debris. In light infections, egg counts may be low and so multiple specimens may be required to confirm their presence. The eggs of *O. viverrini* and *C. sinensis* are similar in size and shape to those of *H. heterophyes* and *M. yokogawai* and cannot be readily differentiated from them. If a patient has not resided in or recently visited areas where infections are endemic, the infection is probably due to *O. viverrini* or *C. sinensis*. The diagnosis can be confirmed by detecting eggs in the bile fluid (duodenal aspirate), by recovering adult worms, or from the clinical history. Like *C. sinensis*, some strains of *O. viverrini* produce eggs that have a comma-shaped appendage at the abopercular end. Differentiation is difficult without a patient history. Multiple egg measurements are usually required to determine size differences; however, absolute identification among the small trematode eggs can be very difficult. Eggs can also be seen in duodenal drainage material or if the Entero-Test capsule is used. Definitive identification usually requires examination of adult worms recovered after therapy or during surgery or autopsy.

Eggs are not found in stool specimens from patients with biliary obstruction, so needle aspiration, surgery, or autopsy specimens may be required to confirm their presence. In these patients, biliary obstruction must be differentiated from enlarged gallbladder, cholangitis with jaundice, liver carcinoma, and cholangiocarcinoma.

Serologic Methods. Elevated IgE levels in serum of up to three or four times normal can be detected in infected patients (29). There is an early rise in the serum levels of IgM followed by elevations of IgA and IgG. In chronic infections, the IgG and IgM levels remain elevated whereas the IgA levels return to normal (30). DNA hybridization and detection of antigen have been used to detect infections in stool (31). DNA hybridization and antigen detection methods have not been extensively tested in areas where infections are endemic but may be slightly better than classical methods of detection (32). Although serologic diagnostic tests for detection of antibodies have been developed, they are not widely available, are not standardized, and give false-positive results due to cross-reactivity with other parasitic diseases (33).

Radiological Screening. Cholangiography, ultrasonography, and liver scans may reveal lesions consistent with liver fluke infection. However, over 75% of patients with this infection tend to have normal ultrasonograms.

Molecular Methods. A single-step duplex real-time fluorescence resonance energy transfer (FRET) PCR merged with melting curve analysis has been developed for the fast detection and differentiation of *C. sinensis* and *O. viverrini* eggs in human fecal samples. *C. sinensis* and *O. viverrini* eggs in infected human fecal samples were detected and differentiated with high (100%) sensitivity and specificity. The detection limit was as little as a single *C. sinensis* egg and two *O. viverrini* eggs in 100 mg of fecal sample. The assay could distinguish the DNA of these two flukes from the DNA of negative fecal samples, fecal samples containing other parasites, and the DNA of human leukocytes. This test would be helpful in determining the accurate range of endemic areas and/or to confirm the co-endemic areas of *C. sinensis* and *O. viverrini* in Asia. This approach also would be valuable for the differential diagnosis of the suspected cases of liver fluke infections among travelers who had visited the endemic countries of these parasites (34). Although this is not the only molecular test, these methods are generally not routinely available, even in endemic areas.

KEY POINTS—LABORATORY DIAGNOSIS

Opisthorchis viverrini

1. Multiple stool specimens may be required to find the eggs.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the small eggs will stain very darkly and resemble debris.
4. Although the wet preparation can be examined using the 10× (low-power) objective, these eggs measure approximately 30 μm and can be easily missed. It is recommended that the 40× (high dry) objective be used for the wet preparation examination.
5. Eggs of *O. viverrini* can resemble those of many other intestinal and liver trematodes.
6. The wet preparation should not be so thick that the small eggs are obscured by normal stool debris.
7. Some strains of *O. viverrini* and *C. sinensis* produce eggs that have a comma-shaped appendage at the abopercular end; this finding differentiates the liver fluke eggs from those of the intestinal flukes *Heterophyes heterophyes* and *Metagonimus yokogawai*.
8. If duodenal sampling is performed (drainage or the Entero-Test), eggs may be found in this material.
9. If available, antigen or antibody detection may be helpful in confirming the infection.

Treatment

In patients with asymptomatic and mild to moderate cases, 75 mg of praziquantel per kg given three times after meals in a single day, or a single dose of 40 mg/kg, is 100 and 90% effective, respectively (18, 19). In those with heavy infections, a single dose of 50 mg/kg produces a cure rate of 97% (3). No eggs are recovered in the stool after about a week; however, clinical symptoms and gallbladder dysfunction may take several months to resolve. Any side effects can be minimized by administration of the single-dose regimen at bedtime.

Mebendazole has also been given at 30 mg/kg/day for 3 or 4 weeks, with cure rates of 89 and 94%, respectively. No adult worms were recovered after therapy (3).

It is important to remember that only 5 to 10% of cases are relieved with praziquantel alone and that relapsing cholangitis and obstructive jaundice may require the use of antimicrobials. For complicated cases, surgery may also be required. Unfortunately, cholangiocarcinoma associated with *O. viverrini* infection carries a poor prognosis.

Epidemiology and Prevention

Dogs, cats, and other fish-eating mammals including humans are reservoir hosts. The infection has been reported to occur in Cambodia, Laos, and Thailand. In Southeast Asia, close to 70% of the population may be infected in some villages. Human infections result from the widely prevalent practice of consumption of metacercaria-infested freshwater fish eaten uncooked, pickled, smoked, salted, or dried. *Bithynia* snails are the first intermediate hosts, and freshwater fish are the second intermediate hosts. The metacercarial cysts are somewhat less resistant to pickling than those of *C. sinensis*; however, thorough cooking of fish is the only guaranteed method of prevention.

It is important to remember that growth of the aquaculture industry must continue to be monitored for problems related to an increased incidence of disease in which infection is transmitted through the ingestion of raw or poorly cooked fish. Freshwater fish aquaculture has increased from an annual production of 136,000 tons in 1952 to 16.6 million tons 50 years later in China, the Republic of Korea, and Vietnam.

Public health education should be used to advise the at-risk population in areas of endemicity about the hazards of eating uncooked fish. This approach has not met with much success because of the difficulty in disrupting established cultural and dietary traditions. Also, defecation in or near ponds or lakes should be prevented, as should the application of night soil where the intermediate hosts are abundant. Community-based therapy of opisthorchiasis will significantly decrease the prevalence of infection; however, therapy alone will not solve the problem because of the high rates of reinfection due to cultural habits. Mass treatment can be undertaken with praziquantel as a single dose of 40 to 50 mg/kg at bedtime.

Opisthorchis felineus

C. sinensis, *O. felineus*, and *O. viverrini* are the three most important liver flukes involved in human health, infecting more than 45 million people. *C. sinensis* and *O. viverrini*, and possibly *O. felineus*, can induce human cholangiocarcinoma as well as inducing other hepatobiliary pathology. Although the three have similar life cycles, only that of *O. felineus* in Europe is predominantly zoonotic, while *O. felineus* in Asia and *C. sinensis* have a mixture of zoonotic and anthroponotic components. Over 680 million people are estimated to be at risk for these infections. *O. felineus* (Siberian liver fluke) infections in humans have been reported from Germany, Kazakhstan, Poland, and the Russian Federation, with the largest area of endemicity being found in western Siberia. Prevalence of human infection with this fluke ranges from 80% in certain regions of Siberia to sporadic cases in the Central and Southern Europe. *O. felineus* infection has been documented in humans and/or animals in 13 countries of the European Union. Over 1.5 million people are infected, with some prevalence rates reaching 95%. The first report of a human infection was in 1892.

Life Cycle and Morphology

The life cycle can be seen in Fig. 19.1. The eggs are elongate and ovoid, with an operculum that resembles that seen in *O. viverrini*. They are generally light yellowish brown and measure approximately 30 by 11 μm. Like the eggs of some strains of *C. sinensis* and *O. viverrini*, some eggs have a small tubercular thickening ("comma") at the abopercular end. Although the miracidium larva is fully developed when the egg is laid, hatching occurs only after ingestion by the snail intermediate host. Apparently, the only known molluscan host is the snail *Bithynia*, in which the first- and second-generation sporocysts and rediae develop. The cercariae leave the snail host and then encyst within the tissues of freshwater fish in the carp family. More than 20 species from 17 genera of freshwater fish serve as second intermediate hosts. Humans, cats, and other reservoir hosts become infected after ingestion of raw or poorly cooked fish. The metacercariae excyst in the duodenum, migrate to the distal bile passages, attach, and begin egg production within 3 to 4 weeks. The adult worm lives attached in the biliary and pancreatic ducts of the host. The adult worm is lancet-shaped and measures 7 to 12 mm in length, which is larger than the adult worms of *O. viverrini* (Fig. 19.4 and 19.7).

Clinical Disease

Although this infection tends to have an incubation period of several weeks, symptoms may appear at the end of the first week. Like infections with other liver flukes, the degree of clinical involvement depends on the extent and duration of the infection. With a low worm burden (up to 50 or 60 worms), there may be localized damage in the distal bile ducts but the liver function remains normal. With a worm count up to 1,000, there may be hepatomegaly and jaundice; the pancreas may also be involved, which can result in digestive problems. Like some of the other liver trematode infections, it is quite likely that chronic infection will lead to cholangiocarcinoma. Overall, the symptoms and clinical sequelae are similar to those seen with *O. viverrini* infection. Acute symptoms can include abdominal pain, urticaria, dizziness, and fever.

In one Italian outbreak with mild infections, the clinical pattern was unclear, and laboratory parameters such as eosinophilia, gamma-glutamyl transpeptidase (GGT), and transaminases often returned to normal or close to normal even when adult worms were still alive in the bile duct and shedding eggs. In this particular outbreak, 21 people with a normal eosinophil count, 16 people with normal GGT values, and 25 people with normal transaminase values shed *O. felineus* eggs 4 to 5 months postinfection. The only laboratory feature that allowed a correct diagnosis was the detection of anti-*Opisthorchis* IgG in the serum, which is detectable for a long period of time and decreases very slowly only after successful treatment (35). Unfortunately, in this situation, the diagnosis was made 5 months after infection, thus confirming that physicians are generally unfamiliar with this disease and may not consider the possibility in the differential diagnosis. In areas where they are not endemic, poor awareness of these liver fluke infections may definitely delay diagnosis and therapy.

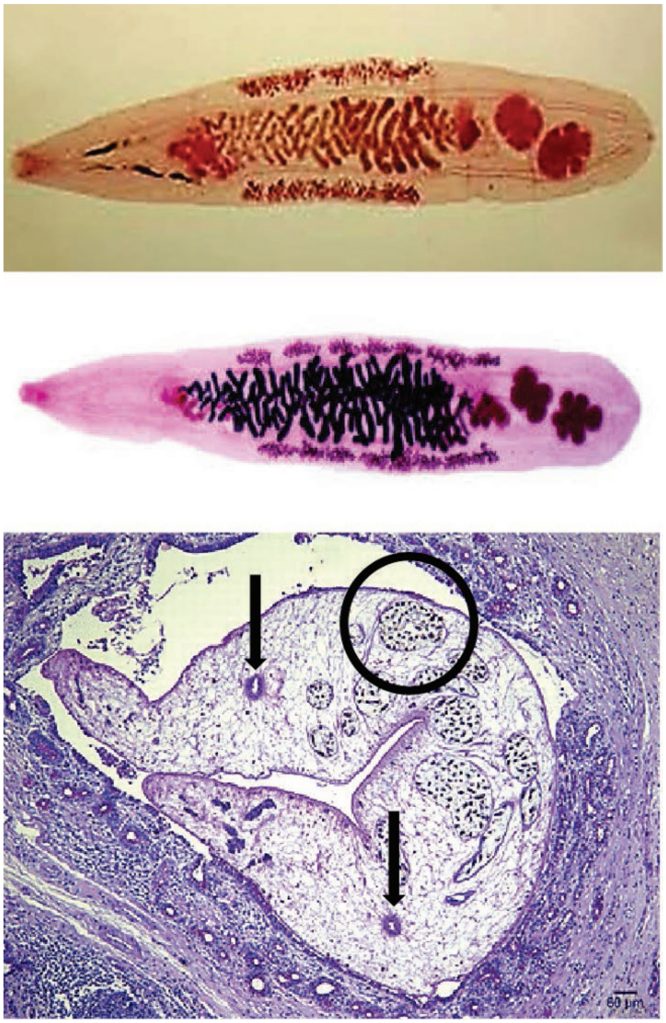


Figure 19.7 *Opisthorchis felineus*. (Top and middle) Adult trematodes (courtesy of the CDC Public Health Image Library). (Bottom) Close-up of *O. felineus*. Multiple branches of the egg-filled uterus are visible (circle), flanked by the paired ceca (arrows) on this cross section through the midbody fluke. Eggs measure 32 by 14 µm on average (length:width ratio of 2.28) and are thus consistent with *O. felineus*. Sparse vitellaria are found on the lateral extremes of the parasite on this section. Slight chronic inflammation of the host's lamina propria without marked eosinophilic infiltrate can be seen. Periodic acid-Schiff stain, original magnification ×100 (courtesy of www.ajtmh.org).
doi:10.1128/9781555819002.ch19.f7

Diagnosis

Fecal Examination. The infection is diagnosed by finding the eggs in the stool; however, they resemble eggs of the other liver trematodes such as *C. sinensis* and *O. viverrini*. Although detection of fluke eggs in stools represents the best way to confirm the diagnosis of opisthorchiasis, it is unreliable in cases with a low worm burden. The eggs have been described as being slightly more narrow (30 by 12 µm) than *C. sinensis* eggs and are more regularly ovoid without a clear shoulder at the operculum. Eggs can also be seen in duodenal drainage material or if the Entero-Test capsule is used.

Serologic Methods. Serology has been performed using an ELISA in accordance with a standard protocol using excretory/secretory (E/S) antigens from adult worms of *O. felineus* maintained *in vitro* (35).

KEY POINTS—LABORATORY DIAGNOSIS

Opisthorchis felineus

1. Multiple stool specimens may be required to find the eggs.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the small eggs will stain very darkly and resemble debris.
4. Although the wet preparation can be examined using the 10× (low-power) objective, these eggs measure approximately 30 µm and can be easily missed. It is recommended that the 40× (high dry) objective be used for the wet preparation examination.
5. Eggs of *O. felineus* can resemble those of many other intestinal and liver trematodes; however, they tend to be somewhat more narrow than the other eggs.
6. The wet preparation should not be so thick that the small eggs are obscured by normal stool debris.
7. Like some strains of *C. sinensis* and *O. viverrini*, *O. felineus* usually produces eggs that have a comma-shaped appendage at the abopercular end; this finding generally differentiates the liver fluke eggs from those of the intestinal flukes *Heterophyes heterophyes* and *Metagonimus yokogawai*.
8. If duodenal sampling is performed (drainage or the Entero-Test), eggs may be found in this material.
9. If available, antigen or antibody detection may be helpful in confirming the infection.

Treatment

A single praziquantel dose of 40 mg/kg after a meal has been recommended. Although side effects are usually mild, they tend to occur in a number of patients and include abdominal pain, vomiting, diarrhea, lassitude, myalgia, headache, and rashes. Any side effects can be minimized by administration of the single-dose regimen at bedtime.

Epidemiology and Prevention

In central, eastern, and southeastern Europe, the Russian Federation, and Kazakhstan, the dog and cat, the red, silver, and polar foxes, domestic and wild swine, rabbit, Norway rat, water rat, beaver, wolverine, marten, polecat, Caspian seal, bearded seal, and lion (in captivity) are infected (33). In some areas of the Ukraine, there is an 82% infection rate, primarily from eating fish on the first day of salting. There is a 15 to 68% infection rate in the middle Urals, with an 84% infection rate in cats. There has been as high as 90% infection with metacercariae in carp in the Tyumen Region of western Siberia (30). Cats are an important reservoir host, with a 43% infection rate being reported from the Ukraine.

Certain factors need to be remembered in understanding this infection and appropriate control measures. Even a single consumption of raw fish dishes can lead to infection. Unfortunately the initial symptoms can disappear quickly, and laboratory test results rapidly return to normal. Symptoms can be nonspecific, and infected individuals can continue to harbor adult worms, often developing serious sequelae years later. Diagnosis is difficult due to a general lack of awareness on the part of physicians, and serologic diagnostic kits are generally not available. Another fact that may be overlooked is that proper freezing of fish to be consumed raw is not always used for the protection of the public (35).

Fasciola hepatica

Fascioliasis (sheep liver fluke disease) is primarily a zoonotic disease that causes liver infections with adult flukes. Over 2.4 million people are infected with this parasite worldwide, including Europe, the Americas, northern Asia, Oceania, Africa, New Zealand, Tasmania, Great Britain, Iceland, Cyprus, Corsica, Sardinia, Sicily, Japan, Papua New Guinea, the Philippines, and the Caribbean. This infection has been recognized by the World Health Organization as an important health problem, particularly in the Andean countries of Peru, Bolivia, and Chile (36). *F. hepatica* was the first trematode to be described and the first for which the entire life cycle was defined; it is interesting that approximately 500 years passed between the description of the parasite and the clarification of its life cycle. The first recorded information on *F. hepatica* infections was provided by de Brie in 1379, when he described the effects of certain types of water plants on sheep that had eaten them. The complete life cycle was established by the investigations of Leuckart and Thomas in 1883, independently of one another (2). The most common definitive hosts are sheep; however, other herbivores such as goats, cattle, horses, camels, hogs, vicuna, rabbits, and deer are often infected as well. In sheep, the migratory phase produces such extensive liver parenchyma damage that the disease is known as liver rot.

Although the disease has a cosmopolitan distribution, at least three cases of autochthonous infection in humans in the United States have been reported (37, 38) (Table 19.1). Two of the patients had eaten watercress at the same time; both had eosinophilia and liver lesions on imaging. The diagnosis was based on serology and both were successfully treated with triclabendazole (38).

Life Cycle and Morphology

Adult worms, which may live for many years in the bile ducts, produce eggs that are carried by the bile fluid into the intestinal lumen and passed into the environment with the feces (Fig. 19.1, 19.4, and 19.8). The eggs are unembryonated, operculated, large, ovoid, and brownish yellow and measure 130 to 150 µm by 63 to 90 µm (Fig. 19.8). The miracidium develops within 1 to 2 weeks in water from 22 to 26°C and escapes from the egg to infect the snail intermediate host, *Lymnaea* sp. These snails are amphibious. Within 4 to 7 weeks, cercariae are liberated from the snail after the production of a sporocyst generation and two or three redia generations. Cercariae encyst on water vegetation, e.g., watercress. Humans are infected by ingestion of uncooked aquatic vegetation on which metacercariae are encysted. Metacercariae excyst in the duodenum and migrate through the intestinal wall into the peritoneal cavity. The larvae enter the liver by penetrating the capsule (Glisson's capsule) and wander through the liver parenchyma for up to 9 weeks. The larvae finally enter the bile ducts, where they mature and produce eggs, which are passed out in the feces. The adult worms can attain a length of 30 mm and a width of about 13 mm and can live for more than 10 years (Fig. 19.4 and 19.8).

Clinical Disease

Acute. The incubation period for fascioliasis can range from a few days to a few months. The patient's symptoms reflect the phase of the infection, as well as the number of parasites present in the host. In the acute phase, symptoms may be present over a period of weeks to months and include dyspepsia, fever, right upper quadrant pain, anorexia, hepatomegaly, splenomegaly, ascites, urticaria, respiratory symptoms, and jaundice. Metacercarial larvae do not cause significant pathologic damage until they begin to migrate through the liver parenchyma. The amount of damage depends on the worm burden of the host. Linear lesions of 1 cm or greater can be found. Hyperplasia of the bile ducts occurs, possibly as a result of toxins produced by the larvae. Symptoms associated with this migratory phase have included fever, epigastric and right upper quadrant pain, intestinal complaints, and urticaria, although other patients remain asymptomatic. Asymptomatic infections appear to be more common in Peru (3). Other symptoms include loss of appetite, flatulence, nausea, diarrhea, and a nonproductive cough. Patients may also have hepatomegaly, splenomegaly, ascites, chest signs, and jaundice. Leukocytosis, eosinophilia, and mild to moderate anemia are found in many patients. Levels of IgG, IgM, and IgE in serum are usually elevated.

Chronic. In the more chronic phases of the disease, the patient generally has few to no symptoms once the flukes have lodged in the biliary passages (Fig. 19.9). However, there may be some epigastric and right upper quadrant pain, diarrhea, nausea, vomiting, hepatomegaly, and jaundice. If the flukes are found in the extrahepatic biliary ducts, symptoms may mimic those seen in cholelithiasis. In the chronic phase, there tend to be some liver function

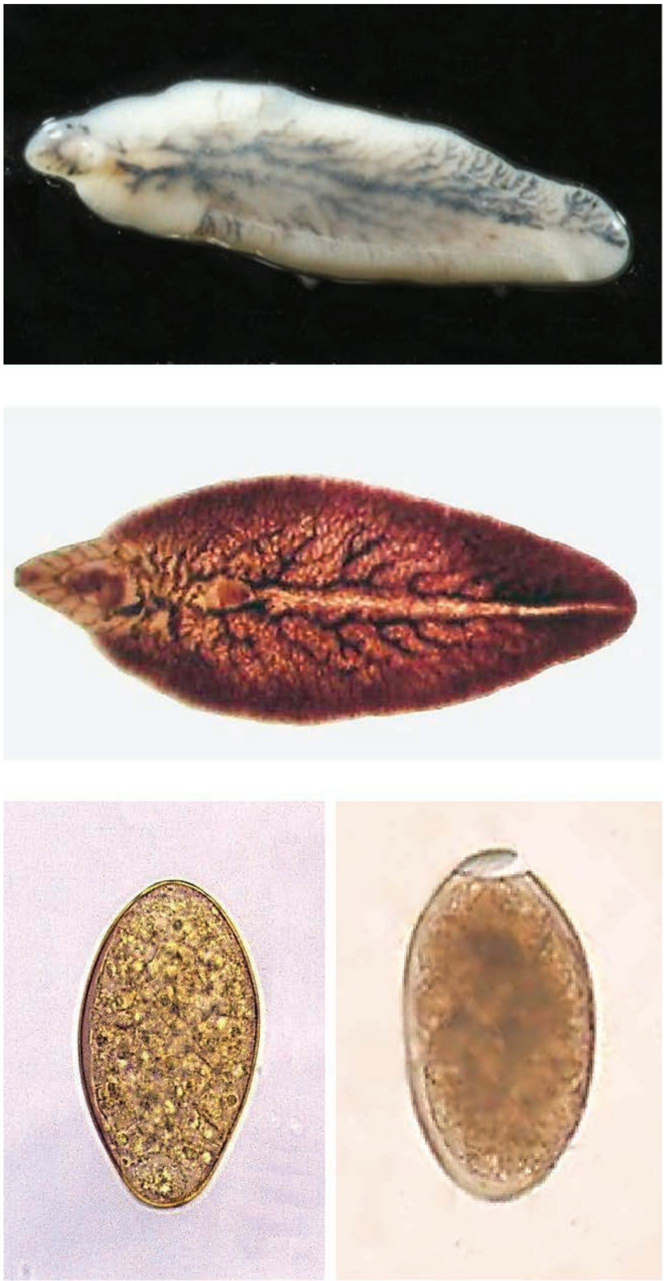


Figure 19.8 *Fasciola hepatica*. (Top) Adult worm, fresh specimen. (Middle) Adult worm, stained. (Bottom) Eggs; note the open operculum on the egg on the right. The operculum can be difficult to see; this egg resembles that of *F. buski* (intestinal trematodes) and *F. gigantica* (liver trematodes), although the egg of *F. gigantica* is larger. The *F. hepatica* adult worm is 20 to 30 mm long by 8 to 13 mm wide. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch19.f8

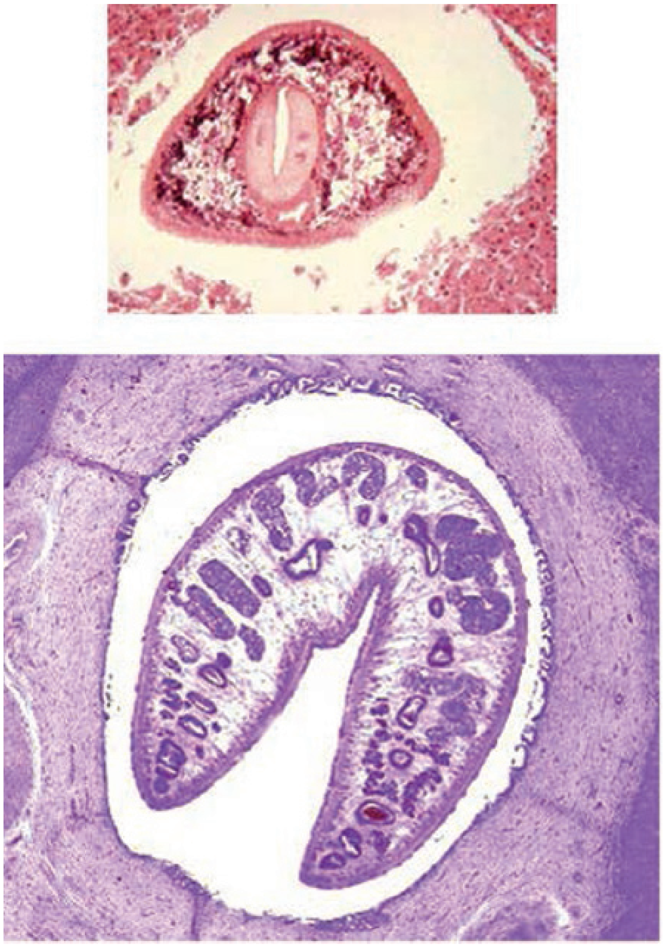


Figure 19.9 *Fasciola hepatica*. (Upper) Adult worm in a bile duct; note the U-shaped worm within the bile duct with surrounding tissues. (Lower) Higher magnification: adult worm in the liver; note the U-shaped worm with surrounding hepatic tissue, with a cellular response seen around the worm. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch19.f9

abnormalities, as well as eosinophilia (Fig. 19.9). Larvae may be found in ectopic foci after penetrating the peritoneal cavity. Worms in human infections have been discovered in many areas of the body other than the liver. Other body sites include the intestinal wall, lungs, heart, brain, and skin. Symptoms mimic those seen with visceral larva migrans and include vague abdominal pain. Unfortunately, surgery may be required to determine the exact cause of the symptoms.

Late. Once the worms have established themselves in the bile ducts and matured, they cause considerable damage from mechanical irritation and metabolic by-products as well as obstruction. The degree of pathologic change depends on the number of flukes penetrating the liver. The infection produces hyperplasia of the biliary epithelium and fibrosis of the ducts with portal or total biliary obstruction. The gallbladder undergoes similar pathologic changes and may even harbor adult worms. Adult worms may reinvade the liver parenchyma, producing abscesses. Symptoms and signs of infection during the late stages after egg production has begun are those associated with biliary obstruction and cholangitis. Acute epigastric pain, fever, pruritus, jaundice, hepatomegaly, and eosinophilia are common.

Halzoun Syndrome. In areas of endemicity where uncooked goat and sheep livers may be eaten, such as Lebanon, adult worms may attach to the pharyngeal mucosa, causing suffocation (halzoun syndrome). This condition is temporary, although it may cause considerable discomfort. The adult worms may lodge on the pharyngeal mucosa, causing edema and congestion of the soft palate, pharynx, larynx, nasal fossae, and eustachian tube. The symptoms include dyspnea, dysphagia, deafness, and occasionally suffocation. It has also been suggested that a number of these cases may be caused by infection with larval linguatulids rather than adult worms of *F. hepatica*.

Diagnosis

Stool examinations and ELISA are recommended for presumptive diagnosis, while radiographic techniques and MRI are used for confirmation and follow-up. Although they may be helpful, invasive techniques such as percutaneous cholangiography, endoscopic retrograde cholangiography, and liver biopsy are not always essential (6). However, liver biopsy may be very important in diagnosing fascioliasis, particularly for asymptomatic disease (3).

Fecal Examination. Several diagnostic methods are available, such as direct fecal smears, Kato-Katz thick smears, Stoll's dilution egg count method, or the formalin-ethyl-acetate technique. The most widely used techniques are the Kato-Katz and formalin-ethyl-acetate techniques, because these two methods have a reasonably high sensitivity at the level of moderate and heavy infections and allow the quantification of infection intensity. The eggs of *F. hepatica*, *Fasciolopsis buski*, *Echinostoma ilocanum*, and *Gastrodiscoides hominis* are similar in size and shape. Differentiation may be difficult without a patient history. Patients may be symptomatic during the first weeks of infection, but no eggs are found in the stool until the worms mature, which takes 8 weeks (39). Multiple stool examinations may be needed to detect light infections.

Spurious Infection. Eggs may be detected in the stools of individuals who have eaten *F. hepatica*-infected liver, thereby yielding an erroneous laboratory result (not a true human infection, but what is called a "spurious" infection). True infection and spurious infection can be differentiated by maintaining the patient on a liver-free diet for at least 3 days. If the patient continues to pass eggs in the stool, the infection is probably genuine.

Serologic Methods. Serologic diagnosis with *F. hepatica* excretion-secretion antigens has been used in areas of endemicity to detect patient antibodies (39). This approach includes ELISA, counterelectrophoresis, and enzyme-linked immunoelectrotransfer blots. Serologic tests can be used to monitor treated patients to ensure successful therapy (40). Serologic cross-reactions have been noted in patients infected with schistosomiasis. Serologic assays are the tests of choice for the diagnosis of acute and chronic fascioliasis when few eggs can be found in the stool or when large populations are to be screened (41).

Radiologic Screening. Imaging methods include ultrasound, CT, MRI, and tissue harmonic imaging. These imaging devices are able to reveal the pathology of the bile ducts. Ultrasound can detect biliary stones, dilatation, and fibrosis due to liver fluke infections, while CT and MRI can determine the lumen diameter of bile ducts, fibrosis, calcification, and epithelial hyperplasia. Tissue harmonic imaging is useful for the observation of bile duct wall trauma and stones.

Molecular Techniques. Various PCR-based methods have been developed and improved to detect egg trematode DNA in stool samples or metacercariae in the second intermediate host. These tests offer high sensitivity and specificity, even in light infections, compared to routine fecal and indirect immunological tests. However, PCR diagnosis is unlikely to become a tool for routine diagnosis in resource-poor areas but remains useful in research.

While research is ongoing in terms of metabolic profiling and biomarkers, it may be years before new diagnostic tests are routinely available. Other considerations include potential sensitivity and specificity, as well as cost and applicability in endemic areas.

KEY POINTS—LABORATORY DIAGNOSIS

Fasciola hepatica

1. Multiple stool specimens may be required to find the trematode eggs. In a very light infection, eggs may not be recovered; however, the patient might be asymptomatic.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the eggs will stain very darkly and resemble debris.
4. The wet preparation can be examined using the 10× (low-power) objective; these eggs are large enough that they can usually be seen using this magnification.
5. Eggs of *F. hepatica* can resemble those *Fasciola gigantica* (liver fluke) and *Fasciolopsis buski* (intestinal fluke).
6. The wet preparation should not be so thick that the eggs are obscured by normal stool debris.

Treatment

Although praziquantel is sometimes effective at a dose of 25 mg/kg taken after each meal for 2 days, it does not appear to be effective in treating patients in Egypt. Treatment with biothionol at 30 to 50 mg/kg on alternate days for 10 to 15 doses is recommended. Triclabendazole at 10 mg/kg as a single dose is also recommended, but this dose may not be as easily obtained. If a single dose has failed, the use of triclabendazole at 20 mg/kg is recommended (16); the drug is given orally in single or multiple doses and has few side effects (40). It acts by inhibiting protein synthesis in *F. hepatica* and will probably become the drug of choice; however, it is not currently available in the United States unless possibly through a compounding pharmacy (18).

Combination therapy in animal models may also lead to other treatment options (21). Artemether and tribendimidine disrupt the tegument of adult trematodes as can be seen via scanning electron microscopy in Fig. 19.10. Prominent morphological features observed following the treatment of rodents with artemisinins included sloughing, blebbing, and roughening of the tegument (Fig. 19.10B and D), while closure of the oral sucker was seen in *O. viverrini* (Fig. 19.10F).

Epidemiology and Prevention

Fascioliasis is a cosmopolitan disease that is found where there is close association of livestock, humans, and snails. The largest numbers of infections have been reported from Bolivia, Ecuador, Egypt, France, Iran, Peru, and Portugal. Although animal fascioliasis is endemic throughout the Americas, human infections are rare in most countries (43). Reservoir hosts include herbivores such as cattle, goats, and sheep. Animal fascioliasis is a major veterinary problem in Europe. The infection is contracted by the ingestion of metacercariae encysted on uncooked water plants, such as watercress. Watercress is the main source of infection; however, in many countries, wild watercress is touted as a healthy natural food. In many areas of the world, animal manure is used as the primary fertilizer for cultivation of watercress.

In surveying potential risk factors in the Northern Peruvian Altiplano, the principal exposure factor for *F. hepatica* infection was drinking alfalfa juice. Human fascioliasis should be suspected when a patient from an area in Peru where livestock is raised presents with recurrent episodes of jaundice and has a history of alfalfa juice or aquatic-plant ingestion or has eosinophilia (44).

Prevention may be accomplished by public health education in areas where infections are endemic, stressing the dangers of eating watercress grown in the wild where animals and snails are abundant. Other measures have included killing snails, treating infected animals, and draining pasture lands.

Fasciola gigantica

Fasciola gigantica is closely related to *F. hepatica*. It is also a common parasite of cattle, camels, and other herbivores in Africa and of herbivores in some Pacific islands. Human infections have been reported in a number of areas of endemicity. Generally, *F. hepatica* is found in temperate zones and is the predominant species in Europe, the Americas, and Oceania, while *F. gigantica* is better adapted to tropical and aquatic environments and is the predominant species in Africa.

Life Cycle and Morphology

Although *F. hepatica* and *F. gigantica* have different geographic distributions, both species essentially share the same life cycle. However, the snail hosts of *F. gigantica* are aquatic rather than amphibious like the first intermediate host for *F. hepatica*. Humans become infected through ingestion of water plants which carry the infective metacercariae. Apparently, developmental stages of *F. gigantica* grow more slowly, survive longer at high temperatures, and are more susceptible to drying than those of *F. hepatica*. The adult worm resembles *F. hepatica* but is somewhat more lanceolate, with a less distinct cephalic cone (Fig. 19.11). The eggs

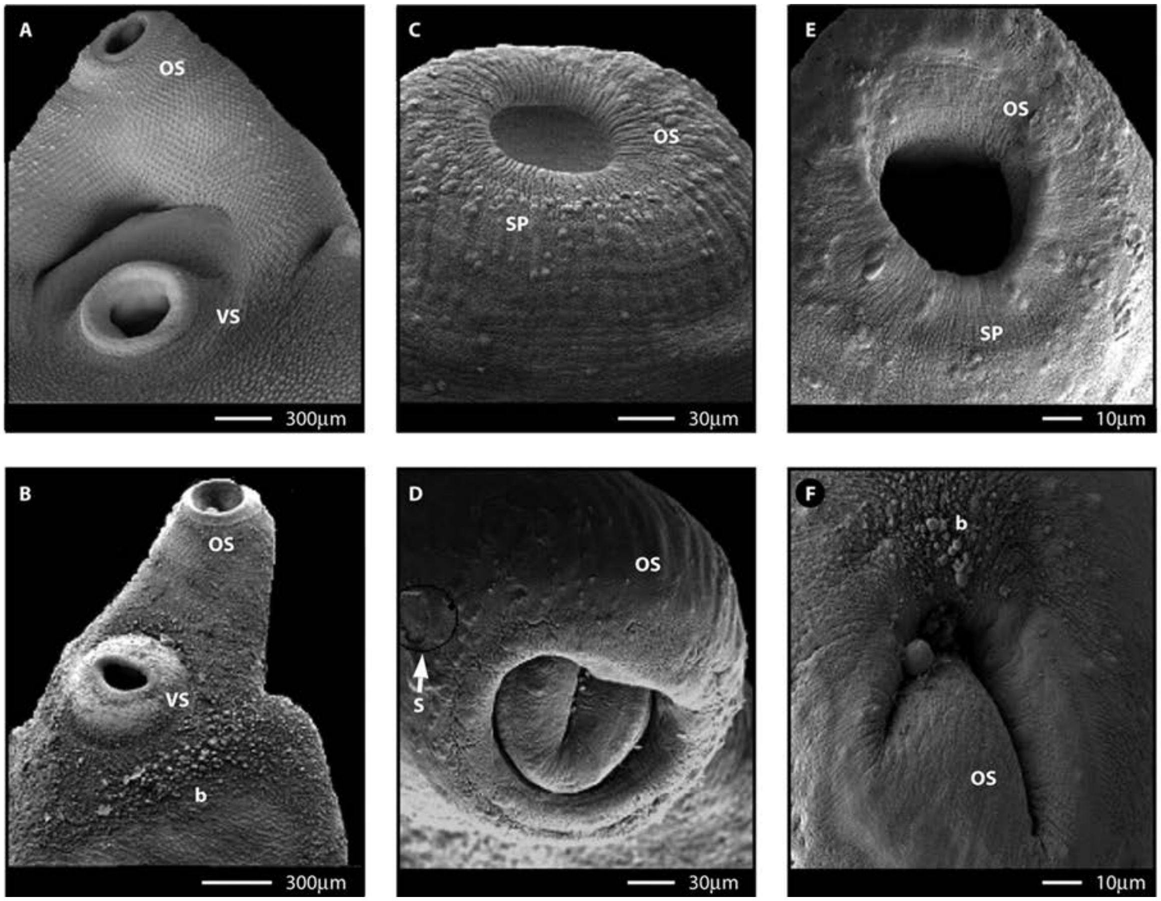


Figure 19.10 (A) Scanning electron microscope observations of the anterior part of an untreated *Fasciola hepatica* depicting the oral sucker (OS) and ventral sucker (VS). (B) A *Fasciola* fluke revealing blebbing (b) near the oral sucker and ventral sucker after administration of a single oral dose of 200 mg/kg artemether. (C) The oral sucker region of a *Clonorchis sinensis* fluke with sensory papillae (SP) visible. (D) Sloughing (s) seen on the oral sucker of an artemether-treated *C. sinensis* fluke. (E) Oral sucker region of an *Opisthorchis viverrini* fluke with sensory papillae. (F) Blebbing and closure of oral sucker observed on an *O. viverrini* fluke treated with a single oral dose of 400 mg/kg tribendimidine. (Courtesy of Keiser J, Utzinger J, *Clin Microbiol Rev* **22**:466–483, 2009.) doi:10.1128/9781555819002.ch19.f10

are very difficult to differentiate from those of *F. hepatica* or *Fasciolopsis buski*; however, they tend to be larger (160 to 190 µm by 70 to 90 µm) (Fig. 19.11).

Clinical Disease

The clinical symptoms of *F. gigantica* infection are very similar to those of *F. hepatica* infection and depend on the worm burden. The prepatent period between infection and the presence of adult worms in the bile ducts is 9 to 12 weeks. Patients may experience fever, nausea, vomiting, abdominal pain, hepatomegaly, hepatic tenderness, and eosinophilia. As in many light trematode infections, there may be vague symptoms or the patient may remain asymptomatic. Abscess or tumor-like reactions have also been reported to occur in subcutaneous tissues or in the liver (3).

Diagnosis

The eggs can be found in the stool; however, they may be absent more often than in infections with *F. hepatica*, so that multiple stool examinations may be required to demonstrate the eggs. Although these eggs are larger than those of *F. hepatica* or *F. buski*, they are very similar in shape. Recovery of adult flukes at surgery would confirm the diagnosis. Other diagnostic options discussed for *F. hepatica* are also recommended for this infection.

KEY POINTS—LABORATORY DIAGNOSIS

Fasciola gigantica

1. Multiple stool specimens may be required to find the trematode eggs. In a very light infection, eggs may not be recovered; however, the patient might be asymptomatic.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the eggs will stain very darkly and resemble debris.
4. The wet preparation can be examined using the 10× (low-power) objective; these eggs are large enough that they can usually be seen under this magnification.
5. Eggs of *F. gigantica* can resemble those *F. hepatica* (liver fluke) and *F. buski* (intestinal fluke); however, they are somewhat larger and could probably be differentiated by careful measurement. Unfortunately, many people might not consider this particular species and thus might not even measure the eggs.
6. The wet preparation should not be so thick that the eggs are obscured by normal stool debris.
7. If available, antigen or antibody detection may be helpful in confirming the infection.

Treatment

Praziquantel is sometimes effective at a dose of 25 mg/kg taken after each meal for 2 days. Treatment with biothionol at 30 to 50 mg/kg on alternate days for 10 to 15 doses is recommended. Triclabendazole at 10 mg/kg as a single dose is recommended, but this drug may not be as easily obtained. If a single dose has failed, the use of triclabendazole at 20 mg/kg is recommended (45). Triclabendazole is not currently available in the United States unless possibly through a compounding pharmacy (18).

Epidemiology and Prevention

Like *F. hepatica*, infection can occur in a wide range of herbivorous mammals when they ingest infected water plants or drink water contaminated with metacercariae. In some areas, the rate of infection in these animal hosts is quite high: in China, the rates are 50% for cattle, 45% for goats, and 33% for water buffalo; in Iraq, the rates are 71% for water buffalo and 27% for cattle; in northeastern Thailand, the rate is 60% for cattle (3). In various surveys, the occurrence of infections in cattle in Zambia was approximately 61%; in Tanzania, the incidence in cattle in traditional, large-scale dairy, and small-scale dairy herds, respectively, was 63, 46.2, and 28.4% (46, 47). Studies in Australia testing a commercially available ELISA for antibody detection have been reported; the results indicate this test will be very valuable in screening cattle and sheep for infections with *F. hepatica* and probably also *F. gigantica* (48).

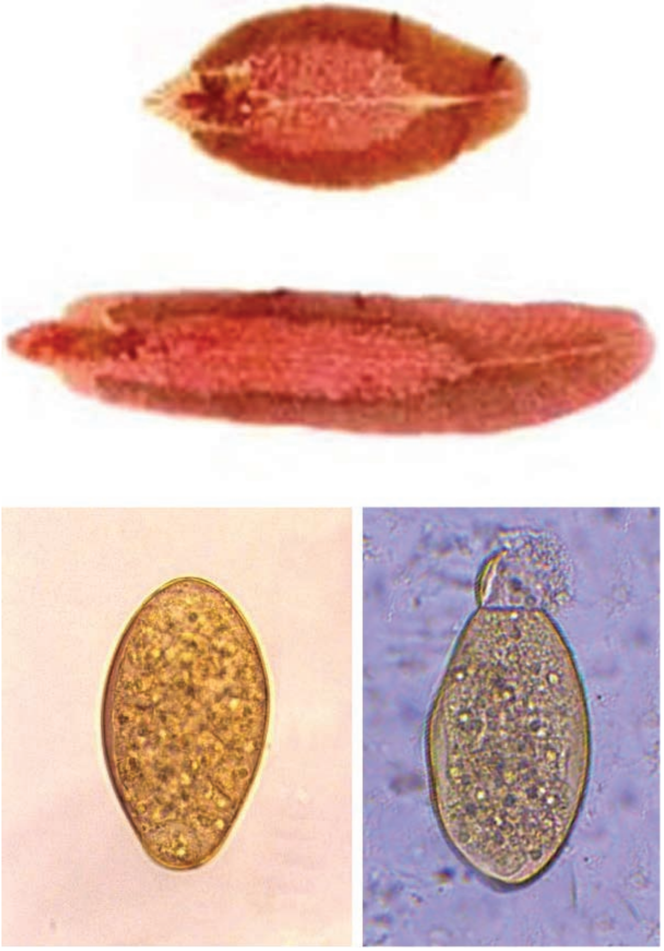


Figure 19.11 *Fasciola hepatica* adult worm (top), *Fasciola gigantica* adult worm (middle), and *F. gigantica* eggs (bottom). The operculum can be difficult to see; this egg resembles that of *F. buski* (intestinal trematodes) and *F. hepatica* (liver trematodes), although the egg of *F. gigantica* is larger (160 to 190 µm by 70 to 90 µm). The *F. gigantica* adult worm is 25 to 75 mm long by 12 mm wide. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch19.f11

Human infections have also been reported in specific areas where animal infection is relatively uncommon; these include Iraq, Hawaii, Uganda, Vietnam, Zimbabwe, Tashkent (Uzbekistan), and some areas within Thailand.

Less Common Liver Flukes

Dicrocoelium dendriticum, *Dicrocoelium hospes*, and *Eurytrema pancreaticum*

Dicrocoelium dendriticum (lancet fluke, lanceolate fluke) is commonly found in the biliary passages of sheep, deer, and other herbivores and omnivores in parts of Europe (e.g., Poland), Turkey, northern Africa, northern Asia, Turkistan (Kazakhstan), parts of the Far East, and North and South America; as an example, in southern Poland, 80% of the sheep are reported to be infected. Although many human infections have been reported, most are probably spurious infections acquired from eating infected raw sheep liver, often causing halzoun syndrome in Lebanon (49). However, true human infections have been reported from Europe, Egypt, Iran, Nigeria, Ivory Coast, and China. Approximately 30 cases of human infection with *D. hospes* have been reported from Democratic Republic of Congo, Ghana, Nigeria, and Sierra Leone. Although *D. hospes* has been thought to be a polymorph of *D. dendriticum*, ultrastructural studies can now distinguish *D. hospes* from other species in the family *Dicrocoeliidae* (50).

Eurytrema pancreaticum is usually found in the pancreatic ducts of hogs in southern China and of herbivores such as cattle, sheep, goats, monkeys, and camels in Asia and Brazil. True infection in humans has been reported in China and Japan.

Life Cycle and Morphology

The life cycle is similar to that of the other liver trematodes. However, in this case, the snail intermediate host is a land snail. The cercariae are released from the snail after rains following a long period of dry weather. They are released from the snail's respiratory chamber as slime balls that are left behind on grass as the snail crawls along the ground or on plants. The ant (*Formica fusca*) is the required second intermediate host for *D. dendriticum* within the United States, while other ants serve this function in other areas. The second intermediate hosts for *E. pancreaticum* are either tree crickets or grasshoppers. Human infection is acquired through accidental ingestion of ants, primarily on fresh herbs or plants used for human consumption. Accidental ingestion of infected crickets or grasshoppers can also result in human infection. The metacercariae excyst and migrate to the biliary passage (for *D. dendriticum*) or the pancreatic ducts (for *E. pancreaticum*), where they then become adult flukes.

The adult worms of *D. dendriticum* are lancet-shaped, flat, and transparent and measure 5 to 15 mm long by 1.5 to 2.5 mm wide (Fig. 19.12). The eggs are thick-shelled, operculate, and deep golden brown and measure 38 to 45 µm by 22 to 30 µm; the eggs of the two flukes cannot be differentiated (Fig. 19.12). The eggs are embryonated when passed and are resistant to drying. The adult worm of *E. pancreaticum* is 8 to 16 mm long by 5 to 9 mm wide and tends to be more ovate and broader than that of *D. dendriticum* (Fig. 19.13).

Clinical Disease

Although the life cycle is similar to that of *F. hepatica*, the pathogenic effects are less severe and patients may report mild symptoms. Symptoms include chronic constipation and flatulent dyspepsia. In patients with heavy infections, there may be jaundice with an enlarged liver. There may also be vomiting and diarrhea, as well as systemic toxemia. Eosinophilia tends to be absent in this infection.

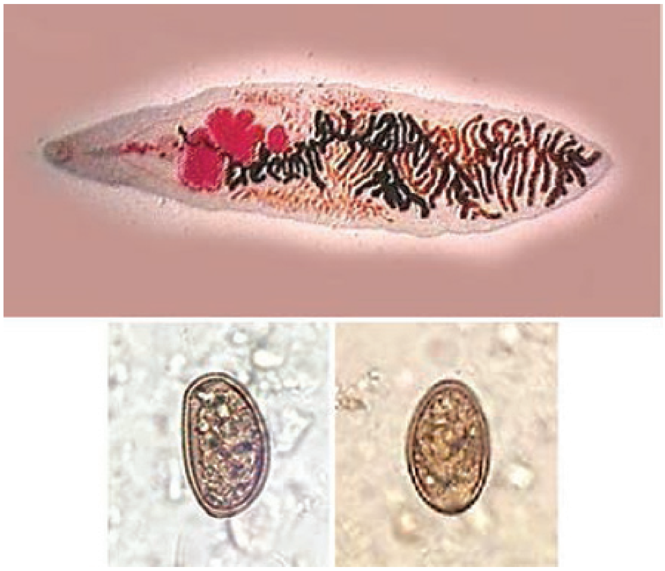
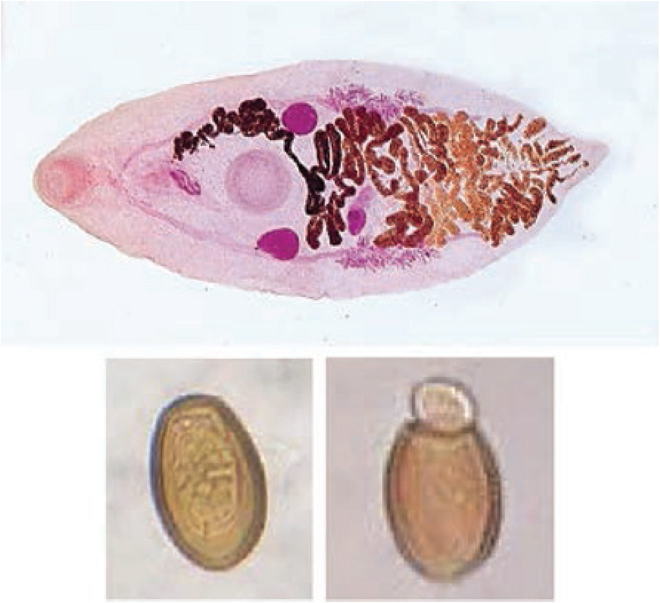


Figure 19.12 *Dicrocoelium dendriticum* adult worm (upper) and eggs (lower). (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch19.f12

Figure 19.13 *Eurytrema pancreaticum* adult worm (upper) and eggs (lower). (Courtesy of http://www.ym.edu.tw/par/html/ParPic/Helminthes/Trematode/Eurytrema_pancreaticum/Eur-pan.htm.) doi:10.1128/9781555819002.ch19.f13



Diagnosis

Diagnosis is based on recovery of the eggs in the stool, bile, or duodenal drainage specimens. It is important to remember that any presumptive true infection must be differentiated from spurious infections acquired from the ingestion of infected raw animal liver. True infection can be distinguished from spurious infection by maintaining the patient on a liver-free diet for at least 3 days. If the patient continues to pass eggs in the stool, the infection is probably genuine. Definitive diagnosis would require the recovery of adult worms at surgery or autopsy.

KEY POINTS—LABORATORY DIAGNOSIS

Dicrocoelium dendriticum*, *Dicrocoelium hospes*, and *Eurytrema pancreaticum

1. Multiple stool specimens may be required to find the eggs; bile and duodenal specimens can also be examined for the eggs.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the small eggs will stain very darkly and resemble debris.
4. Although the wet preparation can be examined using the 10× (low-power) objective, these eggs measure approximately 40 μm and can be easily missed. It is recommended that the 40× (high dry) objective be used for the wet preparation examination.
5. Eggs of *D. dendriticum*, *D. hospes*, and *E. pancreaticum* can resemble those of many other small intestinal and liver trematodes such as *C. sinensis*, *O. viverrini*, *H. heterophyes*, and *M. yokogawai*.
6. The wet preparation should not be so thick that the small eggs are obscured by normal stool debris.
7. If duodenal sampling is performed (drainage or the Entero-Test), eggs may be found in this material.
8. If available, antigen or antibody detection may be helpful in confirming the infection.

Treatment

Praziquantel is the drug of choice, and the same dosage is recommended as that used for infection with *Opisthorchis* infection. Triclabendazole, an imidazole derivative, may also be effective.

Epidemiology and Prevention

Because infection in humans is usually accidental, few preventive measures are effective. Use of wild herbs and grasses as food should be avoided unless they are carefully washed. Crickets or grasshoppers could also be accidentally ingested if they were within plant material that had not been carefully washed.

Lung Flukes

Paragonimus westermani

Paragonimiasis is a disease of humans and carnivores in which adult flukes of the genus *Paragonimus* are found in the lungs. Naterer first detected the lung fluke in 1828; however, Kerbert detected flukes in the lungs of a Bengal tiger in 1878 and named the worms *Distoma westermani*, after the director of the Amsterdam Zoo, Westerman. Both Manson and von Baelz in 1880 reported finding eggs in the sputum of humans, and Yokogawa in 1915 and Nakagawai in 1916 completely described the life cycle. Since 1899, the name *Paragonimus westermani* has been used (Table 19.1). *Paragonimus mexicanus* is an important human pathogen in Central and South America, while *Paragonimus kellicotti* infections are found in North and South America. Most paragonimiasis infections are caused by *P. westermani*, which is the focus of this section (Table 19.2) (51).

Depending on the endemic area, a number of *Paragonimus* species are confirmed or thought to infect humans. These include *P. bangkokensis*, *P. harinasutai*, *P. heterotremus*, *P. hueitungensis* (may be included with *P. skrjabini*), *P. kellicotti*, *P. macrorchis*, *P. mexicanus*, *P. proliferus*, *P. pseudoheterotremus*, *P. siamensis*, *P. skrjabini*, *P. vietnamensis*, and *P. westermani*. As molecular testing expands, the species designations may be changed.

Life Cycle and Morphology

The life cycle is shown in Fig. 19.1. The adult worm is a plump, ovoid, reddish-brown fluke found encapsulated in the lung. Eggs deposited by the worms are ovoid, brownish yellow, unembryonated, and thick-shelled, with an operculum at one end and opercular shoulders. The eggs measure 80 to 120 μm by 45 to 65 μm (Fig. 19.14). *P. westermani* eggs are often confused with *Diphyllobothrium* eggs because they are operculated, unembryonated, and somewhat similar in size. However, unlike *Diphyllobothrium* eggs, *P. westermani* eggs have opercular shoulders and a thickened shell at the abopercular end (Fig. 19.15). Eggs escape from the encapsulated tissue through the bronchioles, are coughed up and voided in the sputum, or are swallowed and passed out in the feces. The eggs hatch in the water in 2 to 3 weeks, releasing a miracidium to infect a susceptible snail host. Cercariae are released after sporocyst and redia generations. Crabs and crayfish are infected by cercariae via the gill chamber or on ingestion of an infected snail. Cercariae encyst in the gill vessels and muscles. Humans are infected by ingesting uncooked crabs or crayfish containing metacercariae (Fig. 19.16). The metacercariae excyst in the duodenum and migrate through the intestinal wall into the abdominal cavity. The larvae migrate around or through the diaphragm into the pleural

TABLE 19.2 Human paragonimiasis

Species	Geographic distribution	Disease (mode of transmission)	Mean egg size (µm) and characteristics
P. westermani[a]	Asia, India, Philippines, New Guinea	Pulmonary (crabs, crayfish, freshwater shrimp)	85–100 by 47; abopercular thickening; oval with barrel-shaped center, operculum with opercular shoulders
P. heterotremus[a]	China, Laos, Thailand	Pulmonary (crabs, raw shrimp salad in Thailand)	86 by 48, uniform thickness
P. mexicanus[a] (*P. peruvianus, P. ecuadoriensis, P. caliensis*)	Mexico, Central and South America	Pulmonary (crabs)	79 by 48; eggshell thin, irregularly undulated
P. africanus	Cameroon, Congo, Nigeria, Liberia, Guinea, The Gambia, and Côte d'Ivoire	Pulmonary (crabs)	90 by 50; tend to be more narrow, abopercular thickening
P. kellicotti	North and South America	Pulmonary (crabs, crayfish)	75–118 by 48–68; shell 2–3 µm thick; abopercular thickening; tapers more sharply than *P. westermani*
P. miyazakii[b]	Japan	Pleural; high frequency of pleural effusion, pleural exudate, marked eosinophilia (crabs, raw juice of crabs, crayfish)	75 by 43; shell very thin; thinner at abopercular end
P. westermani filipinus (*P. philippinensis*)	Philippines	Pulmonary (crabs)	79 by 50; abopercular thickening
P. skrjabini (*P. szechuanensis*)[b]	China	Pleural, subcutaneous nodules (crabs)	75 by 46; egg slightly asymmetrical, small knob at abopercular end
P. hueitungensis	China	Migratory, subcutaneous nodules (crabs); high leukocytosis, elevated eosinophilia	75 by 46; widest part of egg slightly anterior to middle; thin shell, visible opercular shoulders; small knob at abopercular end
P. uterobilateralis	Cameroon, Guinea, Liberia, Nigeria, The Gambia, Gabon, Sudan, Côte d'Ivoire, and southern Africa	Pulmonary (crabs)	70 by 45; abopercular thickening of shell

[a] Pathogenic organisms most frequently isolated from humans; synonyms are given in parentheses.

[b] It has been suggested that in some areas in China and Japan, *P. skrjabini* and *P. miyazakii* should be referred to as *P. skrjabini miyazakii*. Some workers regard the following as synonyms of *P. skrjabini*: *P. miyazakii*, *P. szechuanensis*, and *P. hueitungensis*. Examination of specimens and reclassification studies are under way (51).

cavity and the lungs; they mature to adults in the vicinity of the bronchioles, where they discharge their eggs into the bronchial secretions. Although these worms are hermaphroditic, two worms are usually required for fertilization to occur. The worms can live as long as 20 years, but most die after about 6 years.

Although we generally think of infected raw crabs or crayfish as the most common sources of infection, a number of other foods and cultural practices can be implicated as well, including the ingestion of metacercariae in the flesh of paratenic hosts (Table 19.3).

Clinical Disease

Migration of the larval forms through the intestinal wall into the abdominal cavity is generally not associated with any significant pathologic changes or symptoms. If the larvae remain in the abdomen, some patients may have abdominal pain, intra-abdominal masses, tenderness, fever, diarrhea, nausea, vomiting, and eosinophilia. Once the larvae have reached the peritoneal cavity, they begin to migrate through organs and tissues, producing localized hemorrhage and leukocytic infiltrates.

Pulmonary Disease. When the worms finally reach the lungs and mature, a pronounced tissue reaction occurs, with infiltration of eosinophils and neutrophils. A fibrotic capsule forms around the worm. The cysts contain purulent fluid with flecks or "iron filings" composed of brownish yellow eggs. Many of the cysts perforate into the bronchioles, releasing their contents of eggs, necrotic debris, metabolic by-products, and blood into the respiratory tract. The eggs may also enter the pulmonary tissue, or they may be carried by the circulatory system to other body sites, where they cause a granulomatous reaction (Fig. 19.17, Fig. 19.18).

Symptoms of paragonimiasis depend largely on the worm burden of the host and are usually insidious in onset and mild in patients with chronic infections. Light infections may be asymptomatic, although peripheral blood eosinophilia and lung lesions may be noted on X-ray exam-

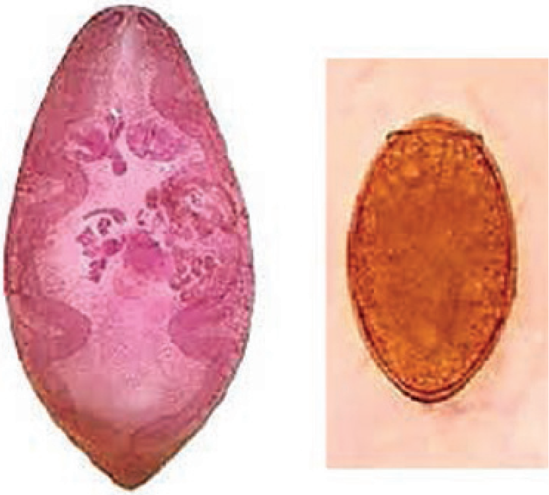
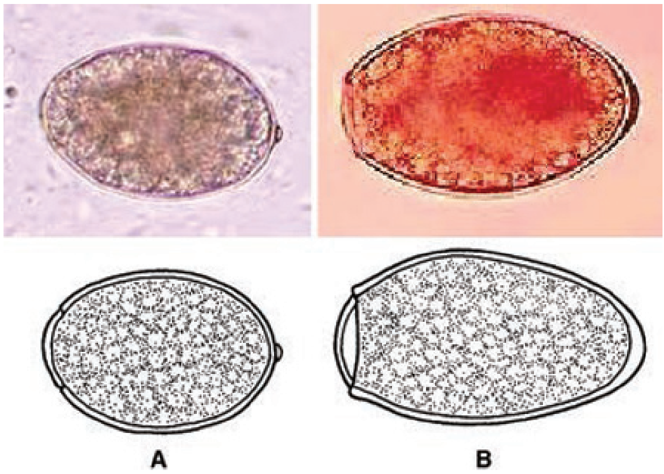


Figure 19.14 *Paragonimus westermani* adult worm (left) and egg (right). The opercular shoulders are clearly visible at the top of the egg, as is the thickened abopercular end. (Left, courtesy of CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch19.f14

ination. As the cysts rupture, a cough develops, with increased production of viscous blood-tinged sputum (rusty sputum, which may have a foul fish odor) and increasing chest pain. The patient may experience increasing dyspnea with chronic bronchitis and be misdiagnosed as having tuberculosis or bronchial asthma (52). The individual generally has a moderately high peripheral blood eosinophilia and leukocytosis, with elevated levels of IgG and IgE in serum. Although some patients exhibit symptoms continuously, others may remain asymptomatic for weeks to months between periods of hemoptysis.

Figure 19.15 Photographs (upper) and drawings (lower) of *Diphyllobothrium latum* egg (A) and *Paragonimus westermani* egg (B). (Illustration by Nobuko Kitamura.)
doi:10.1128/9781555819002.ch19.f15



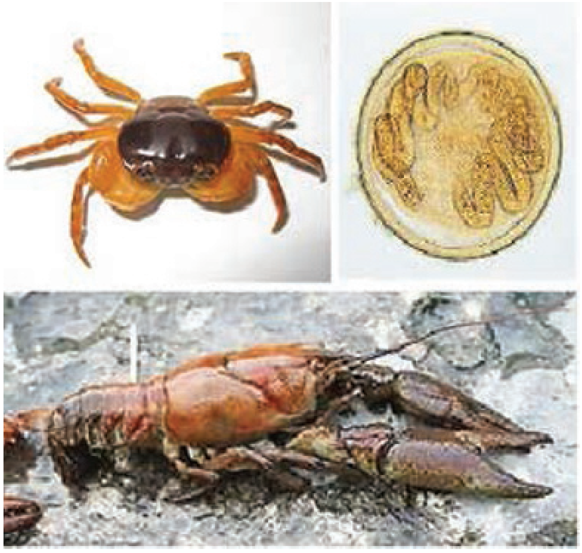


Figure 19.16 Second intermediate hosts for *Paragonimus* spp. (Upper, left) Crab; (right); metacercaria. (Lower) Crayfish.
doi:10.1128/9781555819002.ch19.f16

Cerebral Disease. Larval forms may end up in many ectopic sites other than the lungs; ectopic infections are generally associated with *P. heterotremus*, *P. mexicanus*, and occasion-

TABLE 19.3 Possible means of acquiring infection with *Paragonimus* spp.[a]

Geographic area	Food or cultural practice
Asia	Ingestion of raw or inadequately cooked crabs, crayfish, and shrimp
China	"Drunken crab" (immersion of live crabs in wine for 12 h); raw crab sauce or jam; crayfish curd
Thailand	Kung ten (raw shrimp salad); nam prik poo (crab sauce)
Philippines	Sinugba (roasted crab); kinilao (raw crab)
Korea	Ke jang (crab immersed in soy sauce)
Korea and Japan	Raw juice of crabs or crayfish used in traditional medicine (treatment of measles, diarrhea, and urticaria)
Japan	Ingestion of raw infected wild boar meat (paratenic host)
Africa	Ingestion of raw crustaceans to increase fertility
North America	Ingestion of raw crayfish; on a dare, survival foods, etc.
Central America, South America, Mexico	Several species of freshwater shellfish can harbor this parasite in Brazil; consumption of raw crab with lemon juice (ceviche)
Any of the above	Contaminated utensils, chopping blocks, and cloths used in food preparation

[a] Data from reference 3.

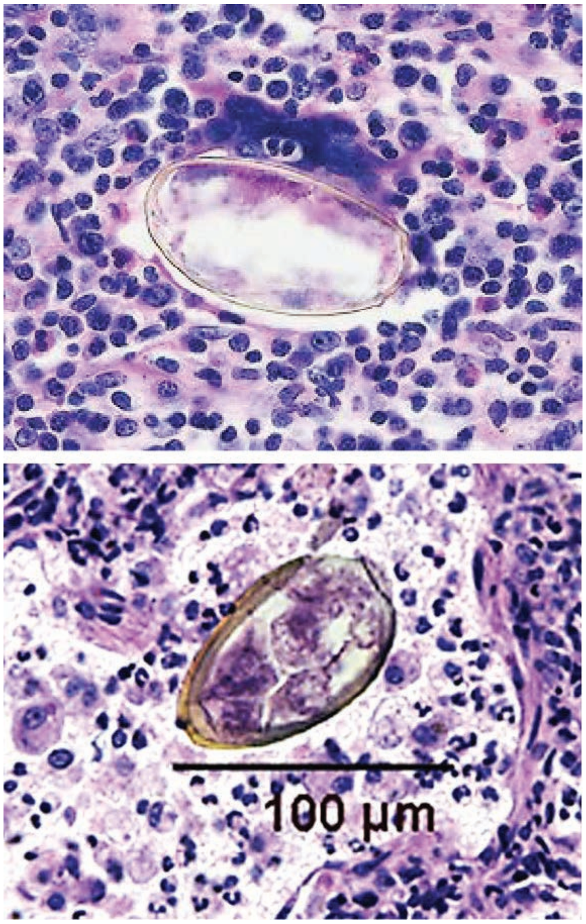


Figure 19.17 *Paragonimus westermani* egg in the lung (cross section). (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch19.f17

ally *P. westermani*. Cysts have been detected in the liver, intestinal wall, muscles, peritoneum, and brain (Fig. 19.19). The most serious consequences of paragonimiasis are the cerebral complications, which are commonly found in younger age groups. Unlike adult flukes in other extrapulmonary sites, worms found in the brain usually contain eggs. The worms probably migrate from ruptured lung cysts and travel through the soft tissues surrounding veins into the brain area. The worms eventually encapsulate, but before being walled off they cause necrosis within the brain tissue, as well as possible cerebral hemorrhage, edema, and meningitis.

Most patients with cerebral or other extrapulmonary lesions have an associated lung lesion or a history of lung disease. Symptoms include fever, headache, nausea, vomiting, visual disturbances, motor weakness, localized or generalized paralysis, and possibly death. More than half of these patients also exhibit personality changes, possible disorientation, and a general decline in cognitive function. Depending on the central nervous system location, symptoms may also include paraplegia, sensory loss, or vision problems. Specific sites can include the cerebral cortex, cerebellum, basal ganglia, medulla oblongata, and spinal cord (53). When a worm dies, the lesion cavity becomes filled with necrotic material. Cerebral paragonimiasis can be difficult to differentiate from brain disease caused by other parasites such as *Schistosoma japonicum*, *Gnathostoma spinigerum*, or *Angiostrongylus cantonensis*, as well as other infectious agents such as bacteria or viruses.

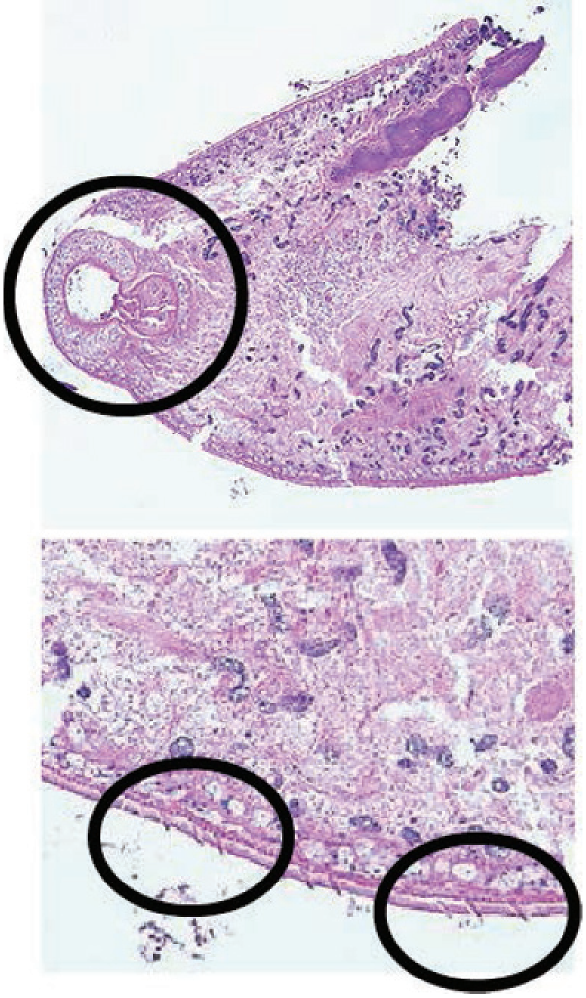


Figure 19.18 (Upper) *Paragonimus* sp. from lung biopsy specimen. Note the presence of the oral sucker (oval). (Lower) Higher magnification showing close-up of the cuticle with cuticular spines (ovals). Both stained with hematoxylin and eosin. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch19.f18

Paragonimiasis in Other Body Sites. Abdominal or subcutaneous masses are frequently seen in patients with infections caused by *P. skrjabini*, *P. heterotremus*, *P. hueitungensis*, or *P. mexicanus*. Migratory subcutaneous nodules occur in 20 to 60% of patients with *P. skrjabini* infection and approximately 10% of patients with *P. westermani* infection. The nodules

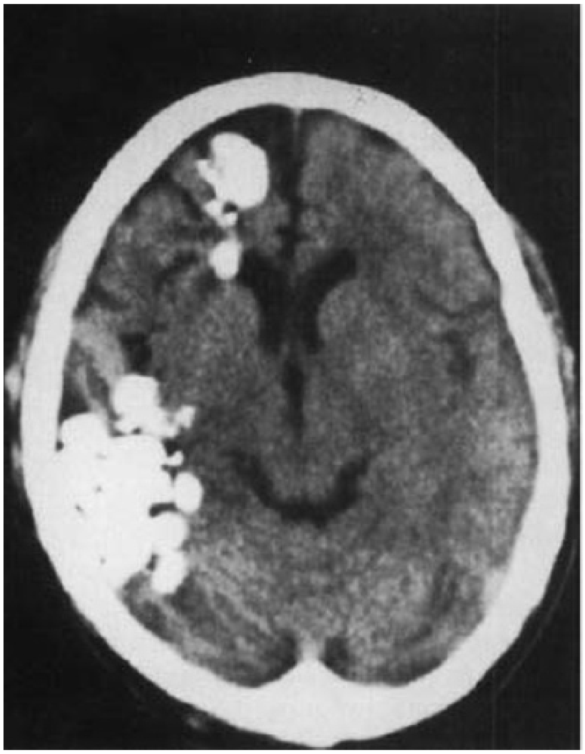


Figure 19.19 *Paragonimus westermani* brain image. CT showing multiple calcified densities in the right frontal and temporal areas. (Courtesy of http://synapse.koreamed.org/DOIx.php?id=10.3347/kjp.2000.38.3.167&vmode=PUBREADER#!po=58.3333.) doi:10.1128/9781555819002.ch19.f19

are firm, tender, a few millimeters to 10 cm, and somewhat irritating. They are often located in the lower abdomen, inguinal region, and thigh and are somewhat mobile. Ulcers or abscesses can also occur in the skin or subcutaneous tissue. Involvement of the mastoid area has been seen in *P. africanus* infections. The skin is one of the common sites for ectopic paragonimiasis which may be seen in children. The typical lesion is a migratory subcutaneous nodule that usually appears on the anterior chest wall, often migrating through the abdominal wall to the pelvic region or lower limbs without causing much discomfort to the patient except mild pain and itching. The abnormal migration is likely to occur in heavy infections in which the immature worm may invade any organ or tissue except bone. Other body sites that have been infected include the breast, lymph nodes, heart, pericardium, mediastinum, kidneys, adrenal gland, omentum, bone marrow, stomach wall, bladder, spleen, pancreas, and reproductive organs. While ectopic lesions are usually thought to be caused by worm migration, dissemination of eggs to other body sites can also be responsible for the pathology.

In China, *P. skrjabini* does not develop to the adult worm stage, but the disease is characterized by the presence of trematode larva migrans (3). These migratory subcutaneous nodules are associated with a high eosinophilia, as well as necrotic liver lesions. Brain involvement is common and is associated with subarachnoid hemorrhage.

Diagnosis

Laboratory Tests. Individuals with symptoms of chronic cough, vague chest pains, and hemoptysis who have resided in an area where infections are endemic and have a history of eating raw crayfish or crabs should be suspected of having paragonimiasis. *Paragonimus* eggs can be detected in the sputum and the stool, and concomitant examinations should be performed to improve the overall detection rate. In many individuals in whom the infection is eventually confirmed, small numbers of eggs are present intermittently in the sputum and feces. For patients with light infections, up to seven sputum examinations have been recommended. Frequently, pulmonary paragonimiasis is misdiagnosed as pulmonary tuberculosis. The Ziehl-Neelsen method for detecting mycobacteria destroys *Paragonimus* eggs (52). It is important to remember that the typical findings of cough, hemoptysis, and eggs in the feces or sputum may be absent in patients with ectopic or pleural *Paragonimus* infection.

Radiography and Scans. Skull radiographs generally reveal round or oval cystic calcifications; they have been described as looking like bubbles. CT scans reveal multilocular cysts with edema, migration tracks, peripheral density, bronchial wall thickening, and centrilobular nodules; ring enhancement may be visible after the use of contrast (Fig. 19.19). MRI demonstrates areas of granulomatous inflammation surrounding the lesions; MRI of the brain may reveal multiple conglomerated iso- or low-signal-intensity round nodules with peripheral rim enhancement. In the liver, CT and MR images may reveal a cluster of small cysts with rim enhancement in the subcapsular area. This finding seems to be characteristic for hepatic paragonimiasis. Solitary nodular lesions can often mimic lung cancer, tuberculosis, or fungal diseases. When a pulmonary mass lesion or empyema is detected in patients who live in areas where paragonimiasis is endemic, paragonimiasis should always be included in the differential diagnosis of lung diseases.

Immunodiagnostic Tests. The various immunological tests which have been evaluated are intradermal test, complement fixation test, immunodiffusion, indirect hemagglutination test, ELISA, dot-ELISA, and Western blot. Complement fixation has been used to diagnose active infections; however, the test becomes negative soon after the death of the worms. An immunoblot assay for the detection of antibodies to *P. westermani* by using adult worm homogenates is highly sensitive and specific (54). ELISA with adult excretory-secretory antigens is very sensitive for detecting parasite-specific IgG and

IgE (55). Pleural effusion fluid is more suitable than serum for detection of infections. The ELISA tests are most widely used for serological diagnosis of paragonimiasis due to their high sensitivity and specificity. However, ELISA tests are more expensive and time-consuming and require costly equipment and experienced persons; frequently, the reagents and antigens are not commercially available. Dot-ELISA has also been used to detect parasite-specific antigen in human sera (56). Monoclonal antibodies used to detect species-specific and stage-specific antigens are highly sensitive and specific for the detection of active infections (57). The serologic assays are available in areas of endemicity or in specialized diagnostic centers. Most of these assays involve nonstandardized reagents and have not been used in clinical trials. An intradermal test is also available for epidemiologic surveys in areas of endemicity; however, positive reactions persist for years after parasitologic cures.

Rapid Test. A dot-immunogold filtration assay (DIGFA) kit was developed in China for anti-*P. westermani* antibody detection. This test is simple and rapid and does not require any special devices and/or experienced technicians; results are obtained within 10 min. This kit was prepared using *P. westermani* antigen and reported in China to have sensitivity and specificity up to 99 and 92%, respectively (58).

KEY POINTS—LABORATORY DIAGNOSIS

Paragonimus spp.

1. *Paragonimus* eggs may be confused with *Diphyllobothrium latum* eggs because of similarities in their size and shape. However, most *Paragonimus* eggs have opercular shoulders and a marked thickening at the abopercular end, unlike *D. latum* eggs.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the eggs will stain very darkly and resemble debris.
4. The wet preparation can be examined using the 10× (low-power) objective; these eggs are large enough that they can usually be seen under this magnification.
5. The wet preparation should not be so thick that the eggs are obscured by normal stool debris.
6. When looking at sputum specimens for eggs, it is necessary to carefully examine any blood-tinged flecks for eggs; the egg clusters have been described as looking like iron filings.
7. In light infections, multiple stool and sputum specimens may be needed before the eggs are detected.
8. If available, antigen or antibody detection may be helpful in confirming the infection.

Treatment

The drug of choice is praziquantel at 25 mg/kg three times at 4-h intervals after meals for 3 days. Very few patients require retreatment; 100% cure is usually obtained except for certain patients with a heavy worm burden. There is dramatic improvement in symptoms, and they are usually gone within a few months. The dose required in cerebral paragonimiasis is generally higher and may have to be adjusted relative to the clinical outcome; convulsions and coma have been seen. These patients should be hospitalized for therapy and should be monitored closely; corticosteroids may also have to be given when treating patients with praziquantel. Bithionol is an alternative drug, which is available under an Investigational New Drug protocol from the Centers for Disease Control and Prevention Drug Service (18).

Triclabendazole at 10 mg/kg as a single dose or two doses is also recommended, but this dose may not be as easy to obtain. Patients should be examined at 3 months to determine the need for retreatment. If a single dose has failed, the use of triclabendazole at 20 mg/kg is recommended (59). Triclabendazole is not currently available in the United States unless possibly available through a compounding pharmacy (18).

Pulmonary paragonimiasis is rarely fatal; however, cerebral disease is characterized by chronic morbidity and symptoms including epilepsy, dementia, and other neurologic sequelae. About 5% of patients with cerebral disease die due to hemorrhage in the first 2 years of the disease (3).

Epidemiology and Prevention

There are approximately 200 million people at risk and 22 million people with paragonimiasis worldwide; a number of species are recognized as causing human disease (52) (Table 19.2). *P. westermani* is the most common human pathogen.

Reservoir hosts include dogs and cats in areas of endemic infection (the Far East and Africa). Eggs expectorated or passed in the feces in the vicinity of lakes or streams where the intermediate hosts live serve as the source of infection. Snail intermediate hosts include *Semisulcospira* and *Brotia* spp. In Ecuador, the intermediate host for *P. mexicanus* is *Aroapyrgus* sp., a freshwater snail belonging to the family *Hydrobiidae* (60). Humans contract the infection through ingestion of raw, undercooked, pickled, or wine-soaked crabs or crayfish; they may also become infected by the ingestion of uncooked meat from wild animals, such as wild boar. The migrating larvae in this meat may pass through the intestinal wall and continue their developmental cycle when eaten. Raw juice from crushed crayfish used as a home remedy for the treatment of measles has been a significant source of infection and may be a cause of cerebral paragonimiasis in children (30).

In most areas, the disease is primarily one of crab- or crayfish-eating mammals, with humans being incidental hosts. Measures devoted to public health education, warning people of the dangers of eating uncooked crabs or crayfish from areas of endemic infection, and sanitary care of utensils and fingers used to prepare food would reduce the risk of infection. Because the cycle depends on animals, the elimination of human paragonimiasis would have little effect on the overall prevalence of the disease.

Paragonimus kellicotti

In North America, paragonimiasis is caused by *P. kellicotti* flukes. Whether *P. kellicotti* extends into Central and South America has not been confirmed. All species of *Paragonimus* have a complex life cycle, requiring snail and crustacean intermediate hosts. Human infections are uncommon, with approximately 20 cases reported to date. However, paragonimiasis due to species not endemic to North America may also be contracted through the consumption of imported food that contains infective metacercariae. Henry Ward and D. S. Kellicott are credited with the independent, near-simultaneous discovery of the only North American *Paragonimus* species in 1894. Ward first described the infection in a cat from Michigan, whereas Kellicott later that year recognized an infection in a dog in Columbus, OH (8).

Life Cycle and Morphology

The life cycle is shown in Fig. 19.1. All species of *Paragonimus* have a complex life cycle, requiring snail and crustacean intermediate hosts. Definitive hosts excrete eggs in feces or sputum, which hatch in water to become ciliated miracidia. The eggs measure 75 to 118 μm by 48 to 68 μm (Fig. 19.20). The miracidia invade soft tissue of snails where they reproduce asexually. Cercariae released from snails invade the secondary intermediate host, a crustacean. Secondary intermediate hosts for *P. kellicotti* flukes are crayfish in the genera *Cambarus* and *Orconectes* (Fig. 19.16). Mammals acquire the infection when they ingest raw or undercooked crustaceans. *P. kellicotti* fluke infections have been found in cats, dogs, bobcats, raccoons, foxes, skunks, minks, and coyotes. Infected raw crayfish are the most common sources of infection.

Clinical Disease

Migration of the larval forms through the intestinal wall into the abdominal cavity is generally not associated with any significant pathologic changes or symptoms. If the larvae remain in the abdomen, some patients may have abdominal pain, intra-abdominal masses, tenderness, fever, diarrhea, nausea, vomiting, and eosinophilia. Once the larvae have reached the peritoneal cavity, they begin to migrate through organs and tissues, producing localized hemorrhage and leukocytic infiltrates. Symptoms include fever, cough, chest pain, dyspnea, night sweats, malaise, abdominal pain, and weight loss. In a series of nine patients, infections were characterized by the consumption of raw crayfish, with alcohol consumption at the time of crayfish consumption being common (61). The median time from symptom onset to the correct diagnosis was 12 weeks (range, 3 to 83 weeks). However, before the correct diagnosis was made, patients received multiple types of unnecessary treatment, some of which were associated with serious illness.

Approximately 75% of patients with this infection demonstrate eosinophilia (6.5 to 40%) and abnormal chest radiographic results. Pleural effusions are also common. Other chest radiographic findings included nodules, opacities, and infiltrates, while chest CT scans showed pleural thickening, pericardial thickening, pericardial effusions, and worm nodules (62). Although eosinophils and Charcot-Leyden crystals may be found in the respiratory secretions and stools of patients with paragonimiasis, these findings are nonspecific. However, these findings suggest an extensive search for fluke eggs should be performed.

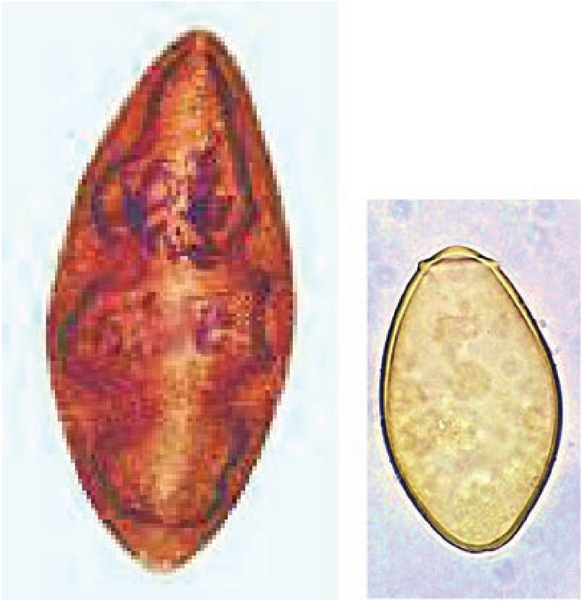


Figure 19.20 *Paragonimus kellicotti*: (left) adult worm; (right) egg. doi:10.1128/9781555819002.ch19.f20

Diagnosis

Laboratory Tests. Individuals with symptoms of chronic cough, vague chest pains, and hemoptysis who have resided in an area where infections are endemic and have a history of eating raw crayfish or crabs should be suspected of having paragonimiasis. *Paragonimus* eggs can be detected in the sputum and the stool, and concomitant examinations should be performed to improve the overall detection rate. In many individuals in whom the infection is eventually confirmed, small numbers of eggs are present intermittently in the sputum and feces. For patients with light infections, up to seven sputum examinations have been recommended. It is important to remember that the typical findings of cough, hemoptysis, and eggs in the feces or sputum may be absent in patients with ectopic or pleural *Paragonimus* infection.

An immunoblot for *P. westermani* flukes performed at the Centers for Disease Control and Prevention (Atlanta, GA) has been reported to be highly sensitive (96%) and specific (99%). However, this assay has not been validated for *P. kellicotti* flukes. In two patients with negative immunoblot results at the Centers for Disease Control and Prevention, these specimens were positive by Western blot with *P. kellicotti* fluke antigen at Washington University (Weil GJ et al., unpublished data). These patients had symptoms and abnormal laboratory test results suggestive of paragonimiasis after ingestion of raw crayfish (61).

Although molecular diagnostic assays of various types have been used for human diagnostic assays, they are not available commercially, nor do we know how they will compare with the traditional diagnostic methods. However, animal research studies are ongoing per the feasibility of using PCR-based methods as potential diagnostic tools for human infections.

KEY POINTS—LABORATORY DIAGNOSIS

***Paragonimus* spp.**

1. *Paragonimus* eggs may be confused with *Diphyllobothrium latum* eggs because of similarities in their size and shape. However, most *Paragonimus* eggs have opercular shoulders and a marked thickening at the abopercular end, unlike *D. latum* eggs.
2. The sedimentation concentration method should be used; because the eggs are operculated, they do not float in the zinc sulfate flotation concentration method.
3. It is important not to add too much iodine to the wet preparation, or the eggs will stain very darkly and resemble debris.
4. The wet preparation can be examined using the 10× (low-power) objective; these eggs are large enough that they can usually be seen under this magnification.
5. The wet preparation should not be so thick that the eggs are obscured by normal stool debris.
6. When looking at sputum specimens for eggs, it is necessary to carefully examine any blood-tinged flecks for eggs; the egg clusters have been described as looking like iron filings.
7. In light infections, multiple stool and sputum specimens may be needed before the eggs are detected.
8. If available, antigen or antibody detection may be helpful in confirming the infection.

Treatment

The drug of choice is praziquantel at 25 mg/kg three times at 4-h intervals after meals for 3 days. Very few patients require retreatment; 100% cure is usually obtained except for certain patients with a heavy worm burden. In general, symptoms, eosinophilia, and radiographic abnormalities should resolve within 1 to 3 months of treatment.

Epidemiology and Prevention

P. kellicotti flukes are believed to be widely distributed throughout the North America. Behavioral factors that may lead patients to eat raw or undercooked crayfish include alcohol consumption, dares, and demonstration of survival skills (42). Although crayfish are commonly regarded as food in survival situations, persons who learn or practice survival skills should be cautioned that eating raw or undercooked crayfish carries a risk for paragonimiasis. Owners and customers of campgrounds and canoe rental businesses should be alerted to thoroughly cook crayfish before eating. The Food and Drug Administration advises cooking shellfish to an internal temperature of 145°F (63°C). Success to date in substantially reducing infections in countries that traditionally have had high incidences of infection has been directly related to educational efforts. These efforts also apply to potential disease prevention within North America.

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20

Blood Trematodes: Schistosomes

Schistosoma mansoni
Schistosoma japonicum
Schistosoma mekongi
Schistosoma malayensis
Schistosoma haematobium
Schistosoma intercalatum

Schistosomes belong to the phylum Platyhelminthes, family *Schistosomatidae*, and are a group of digenetic, dioecious trematodes requiring definitive and intermediate hosts to complete their life cycles. Four species are important agents of human disease: *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, and *Schistosoma haematobium*. *Schistosoma intercalatum* is of less epidemiologic importance. *Schistosoma malayensis* is a member of the *S. japonicum* complex and is found in Peninsular Malaysia; however, the number of cases is small (1). Schistosomiasis affects between 200 million and 300 million people in 77 countries throughout the world and is a significant cause of disease in areas of endemic infections. In Egypt, approximately 20% of the population is infected; prevalence rates in some villages have been estimated to be 85%. The number of infected individuals in China is estimated to be 1.52 million. Although only about 10% of infected people have serious disease, this represents 20 million to 30 million individuals worldwide. Approximately half of the remaining 180 million to 270 million infected individuals have symptoms.

The earliest known instance of schistosomiasis was found in Egyptian mummies of the predynastic period (3100 BCE), using enzyme-linked immunosorbent assay (ELISA) to detect circulating anodic antigen (2). Previously, eggs were detected in the kidneys of mummies from the Twentieth Dynasty (1250 BCE to 1000 BCE). Fujii in 1947 described schistosomiasis caused by *S. japonicum*, and Bilharz in 1951 noted *S. haematobium* infections in Cairo. Schistosome infections in the New World probably began with the African slave trade in the Americas during the 16th and 17th centuries.

Schistosomes are somewhat different from other human trematodes since they (i) have two sexes, (ii) live in the blood vessels, (iii) have nonoperculated eggs, and (iv) have no encysted metacercarial stage in the life cycle. The human is the definitive host for *S. mansoni*, *S. japonicum*, and *S. haematobium*; for *S. mekongi* and *S. malayensis* (both similar to *S. japonicum*); and for *S. intercalatum*. *Schistosoma mattheei*, which causes infections in sheep, cattle, and horses, also infects humans and can cause disease. Other schistosomes have been found in humans but do not tend to cause any pathology. Also, cercariae from birds and mammals can penetrate human skin but cannot complete the life cycle and tend to die without migrating or maturing; however, they do cause cercarial dermatitis.

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Schistosoma mansoni

In one of the earliest reports on African schistosomiasis, Theodor Bilharz first noted the disease but mistakenly described two types of eggs, one with a lateral spine and one with a terminal spine, as occurring in the same adult worm. Manson was the first to note schistosomiasis in the New World, but it was Sambon who adequately described the differences between intestinal and urinary tract schistosomiasis and proposed the name *S. mansoni*. *S. mansoni* was probably established in the Western Hemisphere as a result of the African slave trade.

The prevalence of the hepatitis C virus (HCV) and *S. mansoni* ranges from 1% in Ethiopia to 50% in Egypt. In Egypt, HCV infection, along with *S. mansoni*, is the main cause of chronic liver disease and liver cirrhosis. HCV genotype 4a is highly prevalent in Egypt. Schistosomiasis, HCV, hepatitis B virus, and hepatitis G virus are all associated with liver disease. Coinfection with two or more of these infectious agents may potentiate the pathogenesis of the liver disease (3).

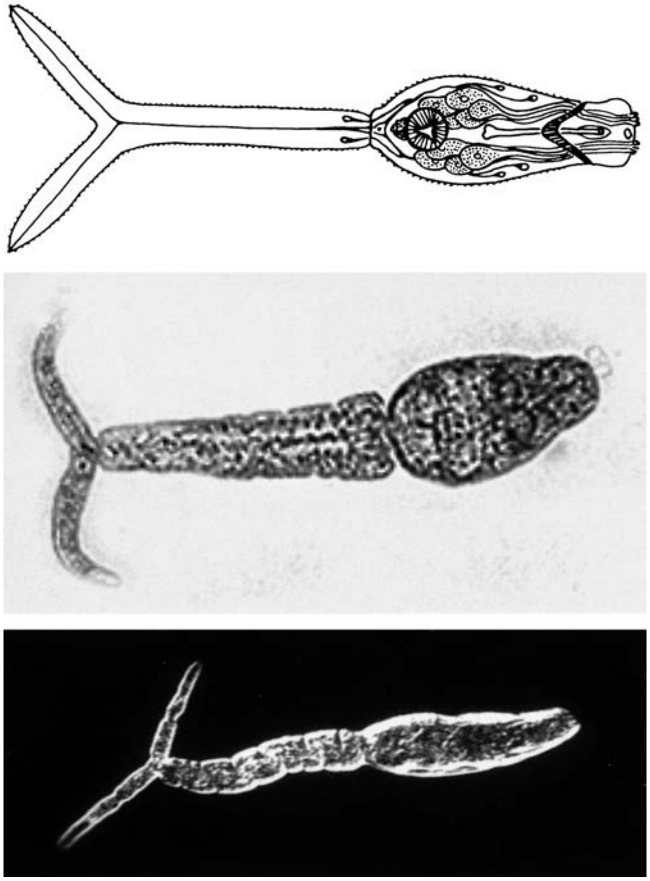


Figure 20.2 Schistosome cercariae. (Top, illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch20.f2

Life Cycle and Morphology

Humans become infected by penetration of cercariae through intact skin rather than by ingestion of metacercariae (Fig. 20.1); penetration can occur within as little as 5 min after initial skin contact. Cercariae consist of a body with glands containing a proteolytic enzyme (elastase), which is used to penetrate skin, and a bifurcated tail that is lost when the cercariae penetrate the skin (Fig. 20.2) (4). Once the cercariae have successfully entered the host, the organism is termed a schistosomulum. After about 48 h, the schistosomulum migrates through the tissues and finally invades a blood vessel. On entry into the blood vessels, the schistosomulum is carried to the lungs and then the liver. Once within the liver sinusoids, the worms begin to mature into adults. The adults of *S. mansoni* are found in the inferior mesenteric veins. The worms form pairs (male and female), with the female lying in the gynecophoral canal of the male (Fig. 20.3 and 20.4). Sexual maturity of female schistosomes depends on the presence of mature male worms. The female worm has selective gene expression in the reproductive tract in the presence of male worms, whereas females separated from male worms lose

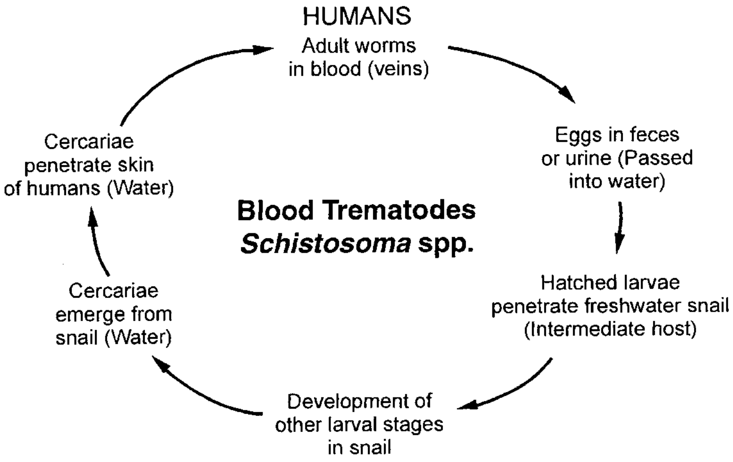

HUMANS
Adult worms in blood (veins)
Eggs in feces or urine (Passed into water)
Hatched larvae penetrate freshwater snail (Intermediate host)
Development of other larval stages in snail
Cercariae emerge from snail (Water)
Cercariae penetrate skin of humans (Water)
Blood Trematodes
Schistosoma spp.

Figure 20.1 Life cycle of schistosomes. doi:10.1128/9781555819002.ch20.f1

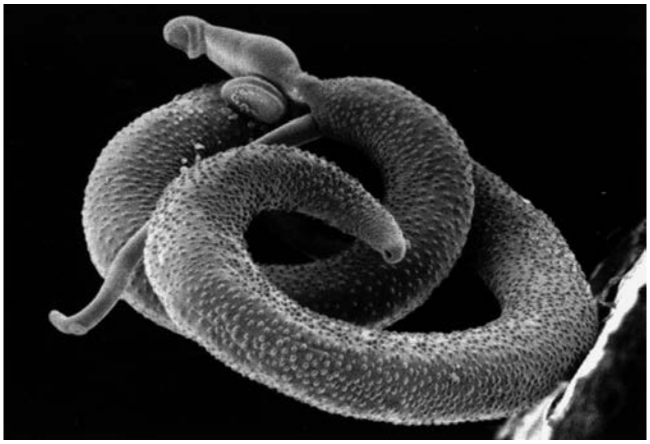


Figure 20.3 Scanning electron micrograph of male and female schistosome *in copula*. doi:10.1128/9781555819002.ch20.f3

this capability. Exploitation of these genetic differences may be of value in drug research and in attempts to reduce host morbidity. Prior to egg production, migration and maturation generally require about 4 to 6 weeks.

Humans are the only important definitive hosts of *S. mansoni* (Table 20.1). Fully embryonated eggs without an operculum (114 to 180 μm by 45 to 73 μm) are passed in the stool (Fig. 20.5 and 20.6). The eggs are light yellowish brown and elongate, and they possess a large lateral spine projecting from the side near one end of the egg. The eggshell is acid fast when stained by a modified Ziehl-Neelsen technique. On reaching water, the eggs hatch, liberating miracidia (Fig. 20.7) which must penetrate a suitable snail host (*Biomphalaria* spp.). Cercariae liberated from the infected snail infect humans when the latter come in contact with cercaria-infested water. A period of 4 weeks or more from the time of first infection with a miracidium is required before the snail sheds cercariae. On infection, the cercariae lose their tails, and the body (schistosomulum) migrates through the lungs to the liver, where it develops to a mature adult in the mesenteric veins of the large bowel. Some worms are also found in the superior mesenteric veins, vesical plexus, and intrahepatic portion of the portal veins.

The adult male and female can reach lengths of 1.2 and 1.6 cm, respectively. They have oral and ventral (acetabulum) suckers at the anterior end. The worm surface is a tegument containing a syncytium of cells. The male worm's body, which is flattened behind the ventral sucker, appears cylindrical as it curves to form the gynecophoral canal to clasp the female worm. The female worm is long, slender, and cylindrical in cross section (Fig. 20.4). While held in the gynecophoral canal of the male, the female ingests 10 times more red blood cells than does the male. The tegument of the male contains many prominent tuberculations, while the tegument of the female is devoid of the tuberculations (Fig. 20.3). The uterus of the female is short and usually contains only one egg at a time. After mating, the female leaves the male and migrates against the flow of blood to the small venules of the intestine. Initial egg production begins 4 to 7 weeks after infection. *S. mansoni* mature females produce 100 to 300 eggs per day.

The eggs are immature when first laid and take approximately 8 to 10 days to develop a mature miracidium. They are nonoperculate and contain a lateral spine. Egg deposition takes place intravascularly. Many of the eggs laid are swept away and become lodged in the microvasculature of the liver and other organs. About half of the eggs swept away by the bloodstream become embedded in the mesenteric venule wall. The presence of eggs in the tissues

Figure 20.4 *Schistosoma mansoni* male and female *in copula*. (Upper) Cross-section (indirect fluorescent antibody stain). (Courtesy of CDC Public Health Image Library/Marianna Wilson.) (Lower) Cross-section of adult worm pair in the mesenteric venule of a mouse; hematoxylin and eosin stain (courtesy of http://openi.nlm.nih.gov/detailedresult.php?img=2154383_pntd.0000032.g002&req=4). Note the female is seen within the male worm in both images (arrows). doi:10.1128/9781555819002.ch20.f4

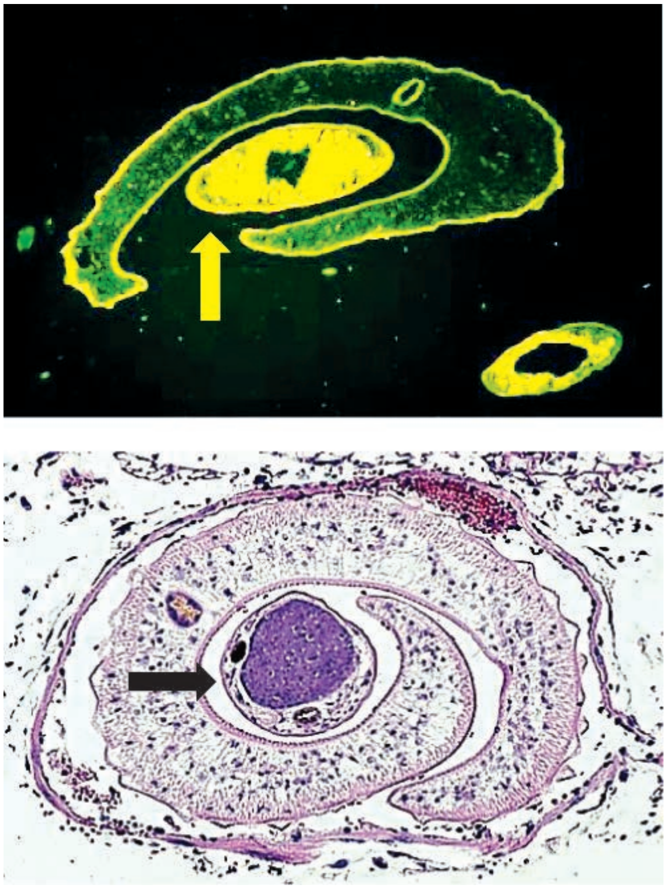


TABLE 20.1 Characteristics of blood trematodes

Species	Distribution	Reservoir hosts	Intermediate snail host genus	Diagnostic specimen or test	Egg		
					Size (µm)	Morphology	Comments
S. mansoni	Africa, Madagascar, West Indies, Suriname, Brazil, Venezuela	Humans, nonhuman primates	*Biomphalaria*	Stool, rectal biopsy, serology	114–180 by 45–73	Elongate, prominent lateral spine; acid-fast positive	In a wet preparation, egg may be turned so lateral spine is not visible; evidence of flame cell activity is proof of viability; can use hatching test (unpreserved specimens) to confirm; occasionally found in urine
S. japonicum	China, Indonesia, Japan, Philippines	Dogs, cats, cattle, water buffalo, pigs	*Oncomelania*	Stool, rectal biopsy, serology	70–100 by 50–65	Oval, minute lateral spine; acid-fast positive	Eggs can mimic debris in the wet preparation; small spine may be very hard to see; debris often clings to the surface of the egg; less likely to be found in urine, but possible
S. malayensis	Malaysia	Jungle rat	*Robertsiella*	Stool	50 by 28	Oval, lateral knob-like structure	Eggs found in liver granuloma; closely related to *S. mekongi*; eggs smaller than *S. mekongi*
S. mekongi	Mekong River basin	Humans, dogs, rodents	*Lithoglyphopsis*	Stool, rectal biopsy, serology	50–65 by 30–55	Subspherical, minute lateral spine	Looks very much like *S. japonicum* egg; lateral spine may be difficult to see
S. haematobium	Africa, Middle East, India, Portugal	Humans	*Bulinus*	Urine, stool (some cases), bladder biopsy, serology	112–170 by 40–70	Elongate, terminal spine; acid-fast negative	If present, usually easy to identify; however, urine sedimentation can be time-consuming; can use membrane filter approach as well; eggs occasionally found in stool; hatching test on urine (unpreserved) sediment is relevant
S. intercalatum	Central and western Africa	Humans	*Bulinus*	Stool, rectal biopsy, serology	140–240 by 50–85	Elongate, terminal spine; acid-fast positive	Egg resembles *S. haematobium* egg but is found in stool rather than urine

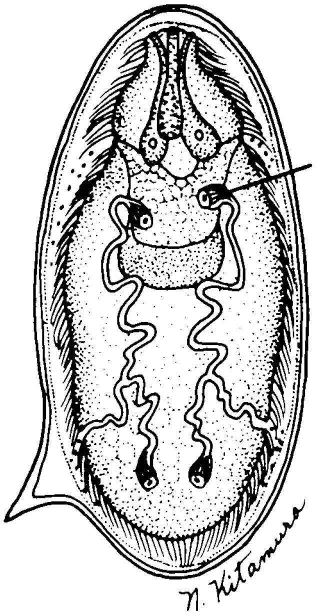


Figure 20.5 *Schistosoma mansoni* egg with a mature miracidium. (Illustration by Nobuko Kitamura; adapted from Beaver PC et al, *Clinical Parasitology*, 9th ed, Lea & Febiger, Philadelphia, PA, 1984.) doi:10.1128/9781555819002.ch20.f5

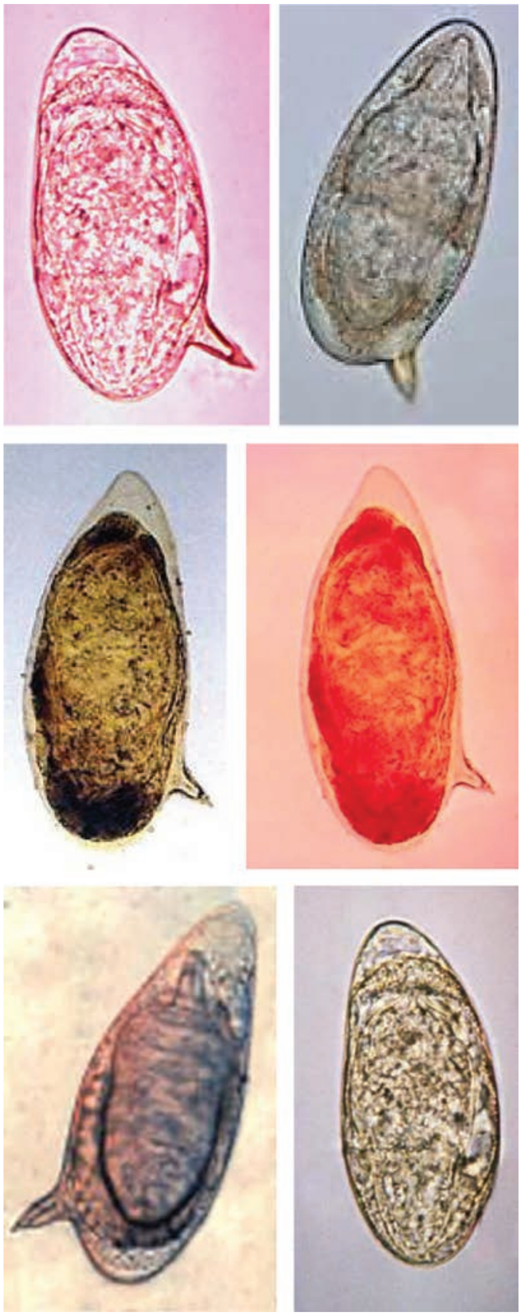


Figure 20.6 *Schistosoma mansoni* eggs. Note the large lateral spines. (Bottom) Note that the lateral spine may not be visible if the egg is turned the wrong way in the wet preparation. Tap on the coverslip to move the eggs into a different position. doi:10.1128/9781555819002.ch20.f6

stimulates granuloma formation; the eggs die, calcify, and are eventually absorbed by the host (Fig. 20.8 and 20.9).

The eggs that are not trapped in the tissues will continue the normal life cycle. The process of maturation and tissue penetration takes about 8 to 10 days; the eggs work their way through the tissues into the lumen of the intestine to be released from the body in the feces. Egg migration through the tissues is facilitated by the release of enzymes by the miracidium through the eggshell. These enzymes help digest the tissue. Eggs making their way through the tissues into the lumen contain a mature miracidium that is released when the eggs are in contact with water (Fig. 20.7). The actively swimming miracidium seeks a suitable snail host, which it penetrates. Within the snail, the miracidium develops into a mother sporocyst, which in turn produces daughter sporocysts. The daughter sporocysts develop in the hepatopancreas of the snail and produce cercariae (Fig. 20.2). The miracidium, which may be either female or male, gives rise to cercariae of the same sex. This portion of the cycle in the intermediate snail host takes approximately 3 to 5 weeks. The cercariae are released from the snail into freshwater, where they may infect humans by penetrating the skin. Stimuli for cercarial shedding of *S. mansoni* cercariae include bright, direct sunlight and water temperatures of 24 to 30°C, leading to higher transmission rates during the summer. *S. mansoni* cercariae move vertically, alternating between migrations toward the surface and sinking. The cercariae attach to the skin of the definitive host via their ventral or oral suckers. Approximately 40% of the cercariae that penetrate the skin will go on to become adult worms; if the cercariae do not find a suitable host, they usually die within 48 to 72 h.

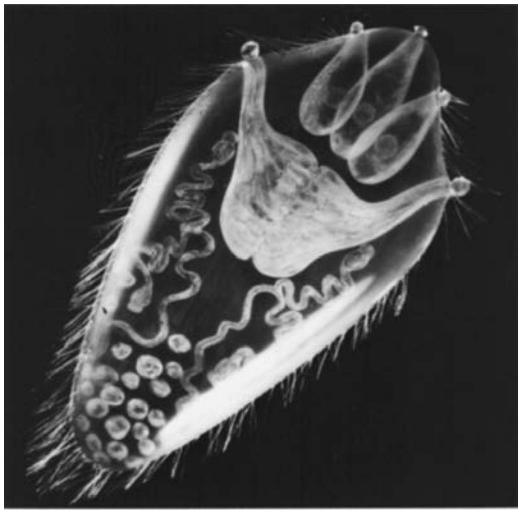


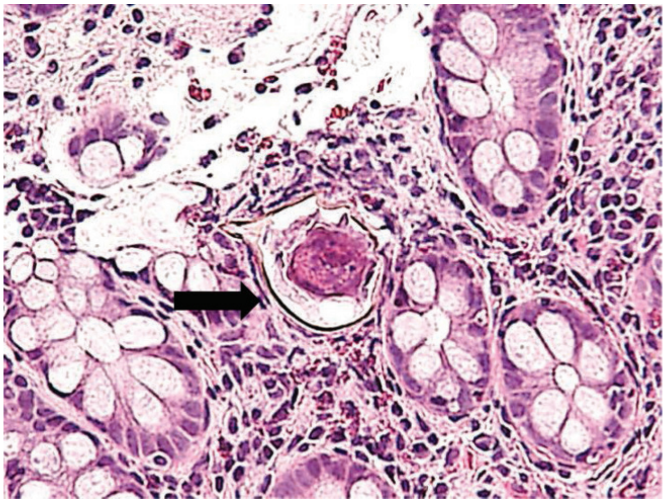
Figure 20.7 *Schistosoma* miracidium (Armed Forces Institute of Pathology photograph). doi:10.1128/9781555819002.ch20.f7

Clinical Disease

Disease syndromes associated with schistosomiasis are related to the stage of infection, previous host exposure, worm burden, and host response. Syndromes include cercarial dermatitis, acute schistosomiasis (Katayama syndrome), and related tissue changes resulting from egg deposition. It has been noted that schistosomiasis exerts disruptive influences on the nutritional reserves and growth of humans from middle childhood through adolescence.

Schistosome Cercarial Dermatitis. Cercarial dermatitis follows skin penetration by cercariae, and the reaction may be due partly to previous host sensitization. Few clinical manifestations are associated with primary exposure, but both humoral and cellular immune responses are elicited on subsequent exposure. After cercarial skin penetration, petechial hemorrhages with edema and pruritus occur. The subsequent maculopapular rash, which may become vesicular, may last 36 h or more. Cercarial dermatitis is common with *S. mansoni* infections. Dermatitis is a constant feature of human infection with avian schistosomes, with cercarial death occurring in the subcutaneous tissues and immediate hypersensitivity reactions occurring at the invasion sites. This condition is known as swimmer's itch. Avian schistosomiasis has been reported worldwide; typically, any bay region with freshwater or saltwater where infected snails are found can be linked to nonhuman cercarial dermatitis. Previous contact with cercariae will lead to a more immediate intense immune response. Schistosomiasis must be included in the list of differential diagnosis of skin lesions, especially in endemic areas, due to the potential sequelae in cases in which the diagnosis and treatment are delayed.

Figure 20.8 *Schistosoma mansoni* eggs in colon biopsy (arrow). (Courtesy of Jennifer Stumph, Spectrum Health; http://www.pathologyoutlines.com/topic/parasitologyschisto.html.) doi:10.1128/9781555819002.ch20.f8



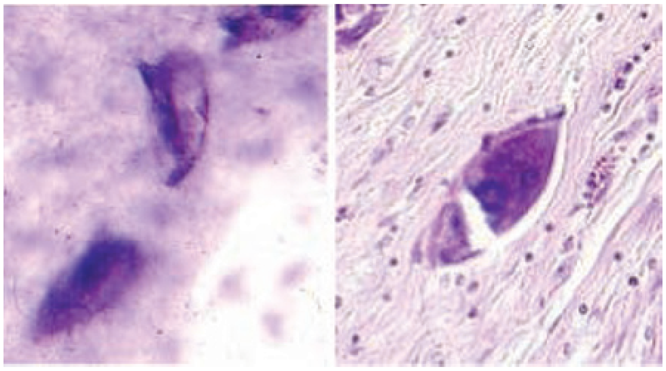


Figure 20.9 Schistosome eggs in tissue. (Left) *S. mansoni*; (right) *S. haematobium*. doi:10.1128/9781555819002.ch20.f9

Acute Disease: Katayama Syndrome. Acute schistosomiasis (Katayama syndrome) is an early clinical manifestation of schistosomiasis that occurs several weeks postinfection with *Schistosoma* spp. (trematode) worms and is associated with heavy primary infections and the initiation of egg production throughout areas of high transmission risk. Because of this time delay and its nonspecific presentation, it is most likely to be misdiagnosed by travel medicine physicians and infectious disease specialists in countries where the disease is not endemic. The signs and symptoms resemble those of serum sickness. Characteristic symptoms include high fever, hepatosplenomegaly, lymphadenopathy, eosinophilia, and dysentery. Diffuse pulmonary infiltrates are seen radiologically, and almost all patients have eosinophilia and a history of water contact 14 to 84 days before presentation of symptoms. This clinical syndrome occurs a few weeks after the primary infection, particularly with *S. mansoni*; however, it is infrequent in inhabitants of areas where infections are endemic. Although widely described, this syndrome does not appear to be as common as it once was, nor are its immunology and pathology well understood (5, 6).

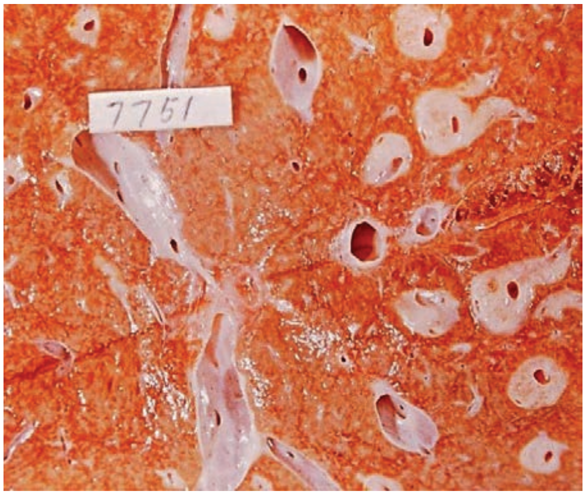
Immune Response. After infection and the transformation of cercariae to schistosomula, the schistosomulum expresses antigens on its surface that evoke a host immune response that provides some degree of resistance to reinfection. As the worms mature, they become less antigenic and more resistant to the immune system-mediated killing mechanisms of the host. The worms are able to incorporate host antigen onto their surface, thus preventing the host from recognizing these parasites as foreign. Because schistosomes are incapable of synthesizing fatty acids or cholesterol, they must bind low-density lipoproteins onto their surface. These bound low-density lipoproteins may be essential in allowing the parasite to evade the host immune response. The adult worms do not appear to cause any damage to the host, nor do they evoke much of an immune response. Immunoglobulin E (IgE) antibodies and eosinophilia appear to play a major role in the host immune response and resistance to reinfection (7). In infected individuals, there is a marked increase in the levels of IgE and IgG4 against various parasitic antigens (8). Individuals develop an age-acquired resistance as they are exposed to infection (9). These antibodies have also been associated with resistance to reinfection. In acute schistosomiasis, the levels of IgA and IgM correlate with the intensity of infection. The detection of IgA might be useful for the differentiation of acute and chronic schistosomiasis. High IgM levels can be detected in both acute and chronic disease.

Chronic Schistosomiasis. After production of eggs by the adult worms (Fig. 20.6), the eggs become trapped in the fine venules and are able to pass through the tissues, escaping into the intestine or, less commonly, the bladder. The eggs liberate a number of soluble antigens, evoking minute abscesses, which facilitate their passage into the lumen. The passage of eggs through the wall of the intestine or bladder leads to symptoms that correlate with the worm burden of the host, including fever, abdominal pain, liver tenderness, urticaria, and general malaise. In *S. mansoni* infection, blood and mucus are detected in the stools and the patient may have diarrhea or dysentery.

Intestinal disease. As eggs are deposited in the tissues, the antigenic substances released by the eggs invoke a host immune response that includes the formation of granulomas around the eggs trapped in the tissues. Many eggs are retained in the intestinal tissue. Cellular infiltrates include lymphocytes, eosinophils, macrophages, and fibroblasts. Granuloma aggregation in the intestinal tract wall is the major cause of pathologic change (Fig. 20.8). The intestinal wall becomes inflamed, thickened, and fibrotic, leading to mechanical obstruction. Apparently, adhesion molecules are important elements in schistosomal intestinal granuloma formation (10). Intestinal schistosomiasis is common in *S. mansoni* infections but can also occur in *S. haematobium* infections.

Hepatosplenic disease. Although many eggs remain where they are deposited, others are swept into the circulation and filtered out in the liver, leading to hepatosplenic schistosomiasis. Hepatosplenomegaly is common in patients with *S. mansoni* chronic infections. In some areas, *S. mansoni*, *S. japonicum*, and viral hepatitis are the most common causes of chronic liver disease. Patients with concomitant viral hepatitis may develop a more severe form of hepatosplenic schistosomiasis. In hepatic schistosomiasis, pathology occurs when schistosome eggs are trapped in the host liver, producing an interleukin 4 (IL-4)- and IL-13-mediated dominant $CD4^+$ Th2 immune response. This response leads to the development of granulomas and fibrosis, with eosinophils, neutrophils, macrophages, hepatic stellate cells, and lymphocytes all identified as major cellular contributors to these sequelae (11). Eggs deposited in the portal triads of the liver stimulate a granulomatous response, leading to continuous fibrosis of the periportal tissue. The fibrotic tissue is white and hard (Fig. 20.10); it has been referred to as Symmer's pipe-stem fibrosis but is now known as fibro-obstructive hepatic schistosomiasis (12). The liver increases in size as a result of the lesions induced by the eggs. The major effect

Figure 20.10 Pipe-stem fibrosis in the liver as a result of *Schistosoma mansoni*. (Original photograph by L. Millman; from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch20.f10



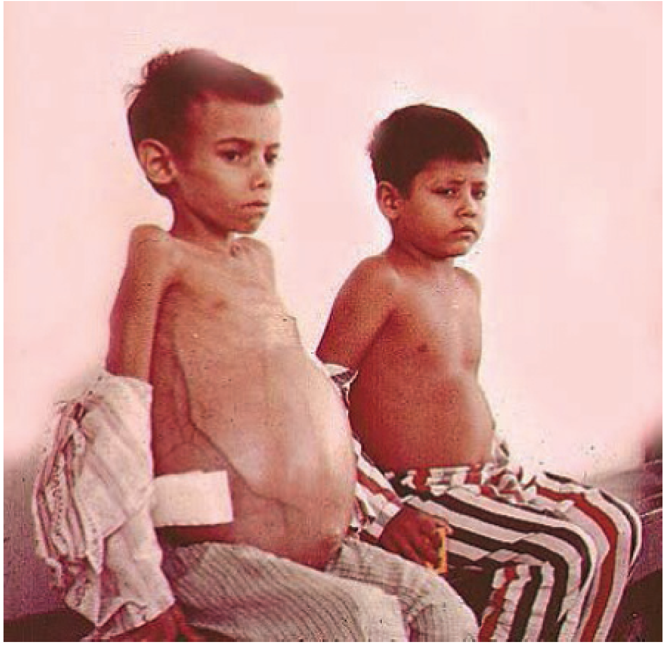


Figure 20.11 Schistosomiasis. Note the engorgement of collateral circulation of the abdomen. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch20.f11

of the liver changes is the blockage of portal blood flow, resulting in increased portal hypertension and the development of collateral circulation, such as esophageal varices (Fig. 20.11). Ascites may be evident, depending on the degree of liver obstruction. The spleen becomes congested and increases greatly in size (Fig. 20.12). The presence of large quantities of antigenic material in the spleen could suggest an important role for this organ in antigen clearance and might provide an additional explanation for the hepatosplenomegaly seen in *S. mansoni*-infected children.

Kidney disease. The level of circulating immune complexes appears to be correlated with the worm burden of the host. These immune complexes may be deposited on the basement membrane and glomerular capillaries, causing nephrosclerosis and kidney failure. The severity of kidney disease is associated with the worm burden and the degree and duration of hepatic fibrosis. The relationship between renal and hepatic disease may be the result of development of a collateral circulation, which would interfere with the normal clearance of circulating antigens and immune complexes by Kupffer cells.

Neuroschistosomiasis. Eggs trapped in the brain have caused focal and generalized seizures. Spinal cord schistosomiasis has been found in patients with *S. mansoni* infections; the myelopathy is probably the result of hypersensitization to the eggs. Myelopathies occur infrequently, and eggs are commonly found in the spinal cord of patients without eliciting symptoms. Neuroschistosomiasis is one of the most severe clinical outcomes associated with schistosome infection. Eggs within the central nervous system (CNS) induce a cell-mediated Th2-driven periovular granulomatous reaction. The mass effect of thousands of eggs and the large granulomas concentrated within the brain or spinal cord explain the signs and symptoms of increased intracranial pressure, myelopathy, radiculopathy, and subsequent clinical sequelae. Cerebral complications include encephalopathy with headache, visual impairment, delirium, seizures, motor deficits, and ataxia, whereas spinal symptoms include lumbar pain, lower limb radicular pain, muscle weakness, sensory loss, and bladder dysfunction (13).

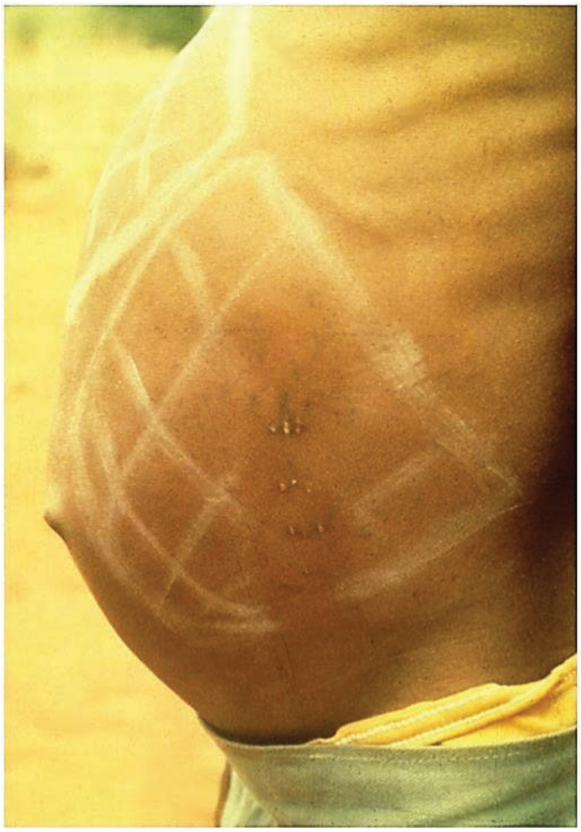


Figure 20.12 *Schistosoma mansoni* infection showing liver and splenic enlargement; 800 worms were removed from this patient. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of R. Goldsmith and B. H. Kean.) doi:10.1128/9781555819002.ch20.f12

Pulmonary disease. Although not frequently described, pulmonary involvement with *Schistosoma* can occur in acute cases and may be much more common than previously suspected. Dry cough is a frequent symptom in patients with Katayama syndrome, but shortness of breath, dyspnea, wheeze, and chest pain may also appear (14). Chest radiographs may reveal nodular lesions with ill-defined borders and less frequently pleural effusion or reticulonodular infiltrates. The most common findings on a chest CT scan are pulmonary nodules from 2 to 15 mm and ground-glass attenuation areas (15). Pulmonary involvement is seen with all forms of schistosomiasis in patients with heavy worm burdens. Schistosomiasis is one of the most common causes of pulmonary arterial hypertension worldwide. Schistosomal lung disease is usually seen only after the development of collateral circulation in infections with *S. mansoni* and *S. japonicum*. The subsequent development of pulmonary hypertension leads to cor pulmonale (right-sided heart failure). Signs of the disease include fatigue, cough with possible hemoptysis, palpitations, dyspnea on exertion, right ventricular hypertrophy, and pulmonary artery dilation.

Bacteremia. Chronic bacteremic infections with *Salmonella* spp. have been associated with *S. mansoni* infections. Common features of this association between *Salmonella* and *Schistosoma* infections include a long history of recurrent febrile illness, frequent isolation of *Salmonella* spp. from the blood, and chronic active schistosomiasis. The *Salmonella-Schistosoma* infection association is seen most frequently in males between the ages of 15 and 30 years. If the underlying schistosome infection is not treated, the *Salmonella* infection will continue to recur. It has also been shown that granulomas of *S. mansoni* in the liver are foci for colonization with *Staphylococcus aureus*, which in presence of staphylococcal bacteremia can form liver abscesses (16).

Diagnosis

We need more accurate, standardized, and sensitive diagnostic techniques for *Schistosoma* infection confirmation. Unfortunately, the development of new assays has evolved slowly, partially because of the current control approach that emphasizes preventive chemotherapy, which is the regular administration of drugs to at-risk populations without prior diagnosis. Another issue involves the perception of inadequate financial returns to the relevant companies that develop these diagnostic products.

Schistosomiasis should be suspected in individuals who have had significant contact with water in areas of endemic infection. Symptoms of acute schistosomiasis include fever, diarrhea, abdominal pain, weight loss, and eosinophilia. Symptoms of chronic disease include chronic diarrhea, abdominal pain, and hepatomegaly or hepatosplenomegaly.

Diagnostic Procedures. Specific diagnosis of schistosomiasis by detection of eggs in stool or urine specimens is possible only after egg production has begun. Eggs may be found in feces as early as 5 weeks after infection. The ease of egg detection depends on the worm burden and the duration of the infection. Patients with a low worm burden or old (chronic) infections may have very few eggs in the feces or urine, and the infections may not be confirmed due to insensitive diagnostic methods. Multiple stools and urine examinations should be performed for any individual suspected of having schistosomiasis. Occasionally, *S. mansoni* eggs are detected in the urine; adult worms may be found in vessels that are not their normal habitat, and this finding is known as "crossover."

S. mansoni eggs are yellowish brown and measure 114 to 180 μm long by 45 to 73 μm wide (Fig. 20.6; Table 20.1). The eggs are elongate and ovoid and have a large lateral spine projecting near one end. Direct detection or concentration techniques can be used to detect eggs in the stool or urine. Direct microscopic examination of stool smears is not very sensitive but may be useful for screening purposes. The Kato thick smear is a simple and sensitive quantitation technique that has been used successfully in the field. In one study, more than 50% of the eggs were missed by the sedimentation technique; the geometric mean egg count was 94 eggs/g when two Kato-Katz smears were used and 43 eggs/g when the sedimentation technique was used (17). However, no details of the centrifugation speed and time were given. Another study indicated that examination of fewer samples collected on different days was more effective than examination of more slides from one stool specimen for accurate estimation of the real infection status.

The zinc sulfate concentration technique is not recommended for schistosome eggs. The eggs rupture and do not float but instead are found in the sediment. The formalin-ethyl acetate technique is recommended for concentrating eggs; however, because it involves fixation, it cannot be used to detect egg viability.

In chronic infections in which the worm burden is light, hatching tests can be performed. Fresh stool specimens are diluted with nonchlorinated water in a sedimentation flask or a beaker. The sides of the flask or beaker are covered with aluminum foil to prevent light from passing through. A light source is used to project a perpendicular light beam through the water at the top. Miracidia that hatch from the live eggs will concentrate in the light and can be detected swimming around. This motility can easily be observed with a hand lens. Aliquots of the surface water can be transferred to a small petri dish and observed under a dissecting microscope for miracidia. Observation periods should be frequent because of the limited life span of the miracidia. Ideally, observations should be made every 30 min over a period of 4 h. The hatching test is designed to mimic the conditions in nature with spring water and sunlight (see chapter 4).

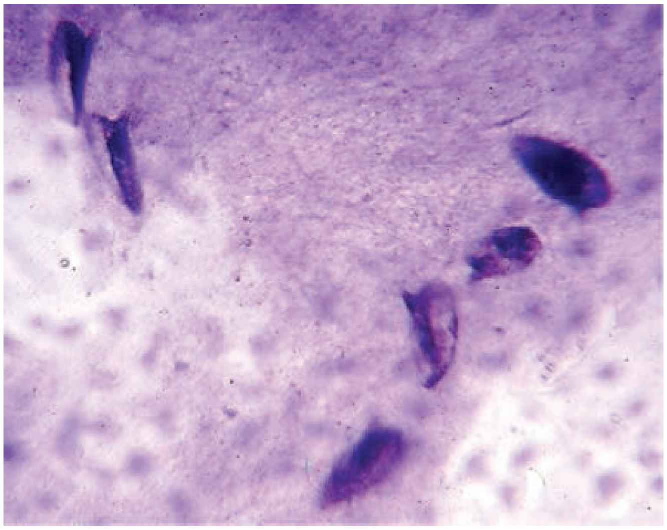


Figure 20.13 *Schistosoma mansoni* eggs in crushed rectal biopsy tissue. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch20.f13

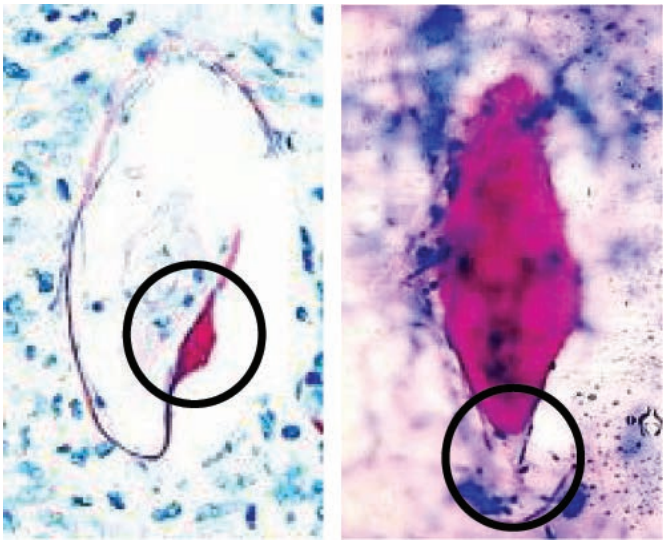
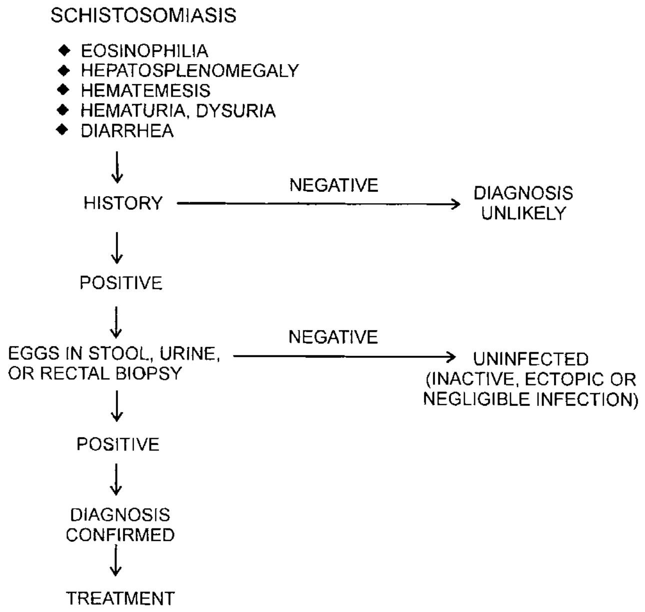


Figure 20.14 (Left) *Schistosoma mansoni* egg shell (acid-fast positive [circle]) (courtesy of http://www.ganfyd.org/index.php?title=Ziehl-Neelsen_staining). (Right) *Schistosoma haematobium* egg. Note the shell is acid-fast negative (circle), but the miracidium larva within the egg shell is acid-fast positive (courtesy of http://parasite.org.au/pugh-collection/Schistosoma%20haematobium%20%201%2022.jpg_Index.html). doi:10.1128/9781555819002.ch20.f14

Biopsy Specimens. Rectal biopsy specimens have been particularly useful in detecting eggs in patients with light, chronic, or inactive infections (Fig. 20.13). The biopsy tissue can be crushed between two glass slides; the tissue can also be stained without routine sectioning. This technique is more effective than histologic examination and allows assessment of the species and viability of the eggs prior to staining. The viability of the eggs can be determined by closely observing the miracidium for flame cell activity. Each miracidium contains two pairs of flame cells, and these are actively beating in live miracidia (Fig. 20.5). Eggs can also be recovered from tissue by digestion in 4% KOH at 37°C for 6 to 18 h and microscopic examination of the sediment. *S. mansoni* eggshells can be stained acid fast with a modified Ziehl-Neelsen stain. This technique has been used in tissue sections to differentiate *S. mansoni* eggs from *S. haematobium* eggs, as the latter are not acid-fast positive. The shell of *S. mansoni*, *S. intercalatum* and *S. japonicum* eggs also stains positive, unlike the eggs of other *Schistosoma* species (18) (Fig. 20.14). As seen in Fig. 20.14, it is also important to remember that while the egg shell may not be acid-fast, the miracidium larva within the shell may be acid-fast (*S. haematobium*).

Antibody Detection. A large number of serologic tests have been used in the diagnosis of schistosomiasis (19, 20); however, many have not been particularly useful because of cross-reactions with other helminth infections, continuation of elevated titers long after successful treatment, and slow immunologic response of the host. Because of the complex life cycle, a large number of antigens have been used in serologic tests. Only a few have been useful. The most frequently used tests are the circumoval precipitin test, the cercaria-Hullen reaction, the indirect fluorescent-antibody test, the indirect hemagglutination test, and ELISA (19, 21). See Algorithm 20.1. The most commonly and widely used methods that have been validated in large-scale field trials include ELISA, the circumoval precipitin test, the indirect hemagglutination test, the indirect immunofluorescence test, and the skin reaction test.

The skin test was used primarily in China during the 1950s; however, this test remains positive for many years after successful therapy and is no longer used. The circumoval precipitin test is based on patient serum precipitation with lyophilized eggs or purified live eggs seen under the microscope. This method is useful for the diagnosis of *S. mansoni* and *S. japonicum* due to its high sensitivity (92 to 100%) and specificity (96 to 100%). However, this test is time consuming (48 h), is complicated to execute, and has a varying period of seroconversion after treatment (20). Using indirect immunofluorescence with adult worm sections, gut-associated fluorescence may occur 44 days after infection and is consistently associated with acute schistosomiasis mansoni infection (Fig. 20.4). However, the need for a costly and complex microscope, experienced personnel, and special reagents limits the efficacy of this technique. The indirect hemagglutination technique uses



Algorithm 20.1 Schistosomiasis. doi:10.1128/9781555819002.ch20.Alg20.1

lyophilized red blood cells coated with crude soluble egg antigen for the detection of positive patient sera through agglutination. This technique can be used in field conditions and requires only basic laboratory equipment.

ELISA methods using soluble adult worm or egg antigens for the detection of antibodies have been used as screening tests, and infection has then been confirmed by the enzyme-linked immunoelectrotransfer blot (EITB) test (19). Individuals infected with schistosomes have elevated IgE and IgG4 responses, as do patients with other helminthic infections. Few studies have investigated the persistence of antibodies over time after therapy (19). It is possible that high titers posttherapy reflect treatment failure and reinfection. Recombinant antigens provide a promising approach for the diagnosis of schistosomiasis, but they are still in the experimental phase requiring validation.

Antigen Detection. ELISA has also been used to detect circulating schistosome antigens in the serum and urine of infected patients and may be one of the preferred methods of diagnosis (22, 23). In addition to diagnosis of infection, antigen levels have been used to assess disease severity in terms of intensity of infection and to monitor the efficacy of therapy (22, 23) (Table 20.2). Individuals treated with praziquantel experienced a rapid clearance of circulating anodic antigens (CAA) and circulating cathodic antigens (CCA); however, in individuals treated with other therapeutic drugs, clearance may take much longer. A dipstick assay based on the anti-CCA sandwich ELISA, on a nitrocellulose format, can detect CCA in the urine of *S. mansoni*-infected patients with a sensitivity of 92%. The results were at least as good as those obtained by ELISA or the Kato-Katz fecal smear examination. This rapid, simple approach may lend itself to more extensive use in future screening programs (24, 25). The reagent strip and cassette formats have proven to work well (26, 27).

Molecular Testing. In human schistosomiasis, the first application of PCR as a diagnostic tool in an area of endemicity was in an assay for *S. mansoni* DNA detection in stool samples. Comparing the PCR assay to stool examinations by the Kato-Katz technique, the PCR assay showed sensitivity of 96.7% and specificity of 88% when applied among 194 inhabitants from an area of endemicity in Brazil. This technique might prove to be useful in low-transmission settings due to its high sensitivity (96.7%) compared to the 79% sensitivity obtained by stool examination. The sensitivity of this technique was reevaluated in other studies in which it was applied to preselected individuals with low worm burden and schistosomiasis-positive patients after treatment (28). Under these specific conditions, both studies confirmed the high sensitivity of the PCR assay. The role of PCR-based assays in schistosomiasis diagnosis outside of research settings remains poorly defined, despite their significant potential in low-transmission settings and in situations where high sensitivity and specificity are required. Research initiatives tend to be focused on the development of point-of-care diagnostics that can rapidly and accurately identify infections in patients, promoting the use of standardized and validated commercial kits and applying molecular high-throughput multiplexing assays for simultaneous diagnosis of helminths and intestinal protozoan infections, particularly for returning travelers at specialized laboratories in the industrialized world.

KEY POINTS—LABORATORY DIAGNOSIS

Schistosoma mansoni

1. Eggs cannot be detected in stool until the worms mature (may take 4 to 7 weeks after initial infection).
2. In very light or chronic infections, eggs may be very difficult to detect in stool; therefore, multiple stool examinations may be required. Biopsy and/or immunologic tests for antigen or antibody may be helpful in diagnosing infection in these patients.
3. The zinc sulfate flotation concentration method should not be used; the eggs do not float. The sedimentation method is recommended.
4. It may be necessary to tap the coverslip to move the eggs; the lateral spine may not be visible if the egg is turned on its side (Fig. 20.6).
5. Occasionally, eggs of *S. mansoni* are detected in the urine (crossover phenomenon).

(continued)

TABLE 20.2 Improved immunologic testing for the diagnosis of *S. mansoni* infection

Test	Comments	Reference
ELISA, IgG, IgM, IgA	Crude adult worm extract as an ELISA antigen provides a serologic method with high sensitivity and specificity for the diagnosis of acute and chronic schistosomiasis and for serologic distinction between the two forms of the disease.	90
Immunomagnetic beads with 1 ml of urine	Assay method uses larger sample volumes than ELISA; enhanced detection can be achieved. Valuable in areas with low prevalence or assessment of cure after therapy.	91
ELISA for cercarial elastase	Cercarial elastase is one of the first proteins released in the host by invading cercariae. Detection of antibodies to this protein indicates exposure but not necessarily active infection.	92
ELISA using egg antigen (CEF6)	In areas of endemicity there was a positive correlation between egg intensity and ELISA value. In other areas, specificity was 90%; false-positive results may have been due to inapparent or cured infection.	93
APIA	Solid-phase alkaline phosphatase immunocapture assay for detection of human IgG antibodies to the alkaline phosphatase of *S. mansoni* adults was evaluated. The method appears to be specific for *S. mansoni* and is useful in areas of endemicity where mixed infections occur.	94
Immunoblot	Sm31/32 protein fraction is highly immunogenic; may be a useful serologic marker for diagnosing and differentiating between acute and chronic infection.	95
Fast dot-ELISA	C5C4 MAb is used for rapid and simple diagnosis; the 63-kDa circulating antigen was detected in 92% of 330 urine samples; the false-positive rate in 130 noninfected individuals was 16%.	96
Indirect hemagglutination combined with ELISA	Erythrocytes coated with *S. mansoni* adult worm antigens and ELISA using *S. mansoni* egg antigens; combined use of these two tests led to high degrees of sensitivity and specificity for *S. mansoni*, *S. haematobium*, and combined infections.	74
Antigen detection in urine or serum	Test provides a quantitatively more stable diagnosis of *S. mansoni* than fecal egg counts based on a single stool exam.	97
Antigen detection in fecal specimens	Compared with the Kato-Katz parasitologic exam, PCR has a sensitivity of 96.7% and a specificity of 88%.	98
Antigen detection in urine using dipstick or reagent strip test	Lateral flow on nitrocellulose strip; detection of CCA; sensitivity equal to CCA ELISA; specificity of 96%.	99
Antigen detection in urine using dipstick	Commercially available antigen capture dipstick; detection of CCA; sensitivity of 83% and specificity of 81%; may be too expensive for wide use (US $2.60).	24
Antigen detection in urine using reagent strip test	Under field conditions, 83% sensitivity, 81% specificity for *S. mansoni*	26
Antigen detection in urine using urine-circulating cathodic antigen cassette	89% sensitivity, 100% specificity; in moderate and high-prevalence setting very effective	27

KEY POINTS—LABORATORY DIAGNOSIS

Schistosoma mansoni (continued)

6. Patients who have been treated should have follow-up ova and parasite examinations for up to 1 year to evaluate treatment.
7. In active infections, the eggs should contain live or mature miracidia. Examination for the confirmation of flame cell activity must be performed with fresh specimens, using the microscopic wet mount test or the hatching test; no preservatives can be used prior to these two tests.
8. During the hatching test, the light must not come too close to the surface of the water, since excess heat can kill the liberated larvae. Also, the water should be examined about every 30 min for up to 4 h.

Treatment

Praziquantel. Praziquantel, a pyrazinoquinolone, is the drug of choice for treating schistosome infections (29). The current recommendation is 40 mg/kg/day in two doses for 1 day. Praziquantel can also be taken orally in a single dose and is well tolerated by the host. Side effects, which are usually mild and transient, include abdominal discomfort, dizziness, drowsiness, headache, fever, and loose stools. These effects usually disappear within 48 h. In heavily infected individuals, the side effects, although transient, may be very severe (30). Split doses have been recommended for treating heavy infections, but this may be a concern for population-based therapy, where individuals may not present themselves for follow-up therapy. Data suggest that a single dose of praziquantel (40 mg/kg of body weight) may have a longer-lasting effect than previously thought, possibly more than 2.5 years (31). Praziquantel has little effect on immature stages of the parasite, and this affects the cure rates as the immature parasites develop to maturity and egg production. The mechanism of action may involve glutathione *S*-transferase, a detoxifying enzyme found in many helminths. Cure rates have been reported to be from 70 to 95%. Praziquantel damages the tegument of the worm, thereby exposing the antigens to parasite-specific antibodies. The use of immunoglobulins along with praziquantel has been proposed to increase the efficacy of treatment.

Praziquantel is rapidly taken up through the gut (in humans more than 80% of the orally administered dose is absorbed from the gastrointestinal tract) and reaches its maximal plasma concentration within 1 to 2 hours. Although the exact mechanism by which praziquantel kills the parasite is not well understood, it is known that the immune response of the host (granulocytes, histiocytes, antibodies) plays an important role in drug efficacy. It is possible that disease states of humans that lead to immunodeficiencies, such as infection with HIV-1, may render praziquantel less effective in treating schistosomiasis. However, studies indicate that persons with HIV-1 infection can be treated effectively for schistosomiasis with praziquantel (32).

Repeated praziquantel therapy can lead to improvement of liver morbidity and prevention of hepatic fibrosis, even in a well-established focus of hyperendemic infection. These results were based on a parasitologic, clinical, and ultrasonographic longitudinal study in a focus of hyperendemic *S. mansoni* infection. Support is also emphasized for programs that encourage people to seek examination and treatment on their own. This type of chemotherapy-based control strategy is called passive chemotherapy.

Reduced efficacy of praziquantel in treating human *S. mansoni* infections and infected animal models has been reported (33, 34). Drug resistance to praziquantel has been demonstrated in the murine model, and there have been unsubstantiated field study reports of resistance. However, resistance to praziquantel in schistosomes is now considered a fact in strains selected in the laboratory. In at least two human populations in Senegal and Egypt with endemic infections, resistance or tolerance to praziquantel has been confirmed. Although the extent of the problem is unclear, it is important to determine the prevalence and significance of the problem and to develop alternate control strategies.

Oxamniquine. Oxamniquine, a nitroquinolone, is active only against *S. mansoni* infections and is very effective against the strains in the Americas and West Africa when given at 15 to 20 mg/kg of body weight. North and East African strains of *S. mansoni* are less sensitive to this drug, and doses of up to 60 mg/kg given over 2 to 3 days may be required to achieve the same results. The drug is more active against male than female worms and has little effect against immature worms. Side effects include abnormal electroencephalograms and dizziness. Oral treatment has reduced egg counts by 95%, with 70 to 100% cure rates. Despite being tolerant to praziquantel, a Senegal isolate of *S. mansoni* was fully susceptible to oxamniquine (35). Unfortunately, this drug is relatively expensive, making it more difficult to use in control programs, particularly if a higher dosage is required. Although mass therapy with this drug can lead to reduction of schistosomiasis morbidity, approximately 68% of patients do not show improvement in their condition (36). It is also important to remember that oxamniquine is contraindicated in pregnancy.

Summary. Patients who have been treated for schistosomiasis should be monitored posttherapy to determine the effectiveness of the treatment. Depending on the drug, follow-up stool or urine examinations may be needed for as long as 1 year posttherapy. It is not safe to assume that one course of therapy or disappearance of symptoms is indicative of cure. Optimal posttreatment reevaluation times have not been established. Patients, particularly those who were heavily parasitized, should be monitored for a reduction in the number of excreted and viable eggs within a few months of therapy.

For patients with secondary bacterial infections, appropriate antibiotics may also be needed to treat systemic infections. Patients suffering from severe Katayama syndrome and transverse myelitis may require steroid treatment. A complete review of the management and therapy of schistosomiasis can be found in Table 20.3.

Epidemiology and Prevention

S. mansoni infections occur in western and central Africa, Egypt, Madagascar, and the Arabian Peninsula. As a consequence of the African slave trade, the infection was established in the Western Hemisphere in Brazil, Suriname, Venezuela, and the West Indies. Human infections are nearly all derived from human sources, even though nonhuman primates, insectivores, marsupials, and rodents can carry the infection. The infection is transmitted by *Biomphalaria* sp., an aquatic snail.

The geographic distribution of the disease depends on the distribution of the intermediate snail hosts and the opportunity to infect humans and snails. Schistosomiasis is transmitted from infected people defecating or urinating in or near water where the appropriate snail host resides. Infections can persist indefinitely in humans. Most infected individuals have low worm burdens, but a few have very heavy infections. It is this latter group that probably makes the greatest contribution to the dissemination of schistosomiasis. The highest rates of infection have been found in

TABLE 20.3 Management and therapy of schistosomiasis[a]

Condition or disease manifestation	Approach	Comments
Schistosome cercarial dermatitis	Self-limited and may go undiagnosed; in severe cases, topical corticosteroids and parenteral antihistamines reduce symptoms	Other names include swimmer's itch, schistosome dermatitis, clam digger's itch, sawah itch, and koganbyo; symptoms will vary due to host susceptibility, schistosome type, and patient's previous exposure history.
Katayama syndrome	Corticosteroids for life-threatening disease; therapy according to schistosome species	Tourists who returned home within a month of exposure; history of water contact, possible swimmer's itch, serum sickness-like disease are clues for possible schistosomiasis.
Gastrointestinal disease	***S. mansoni*:** Praziquantel (40 mg/kg) single dose or oxamniquine (15 mg/kg) single dose for adults (20 mg/kg for children); North and East Africa (60 mg/kg) over 2–3 days in 3 or 4 equally divided doses ***S. japonicum*:** Praziquantel (60 mg/kg) 2 or 3 doses in a single day	Many complications of intestinal granulomas and/or polyp disease are the same as for inflammatory bowel disease (Crohn's and ulcerative colitis); misdiagnosis could have serious sequelae due to potential use of steroid therapy.
	Colonic polyps: Colonoscopy to confirm extent of disease; chemotherapy; surgery may be required for fibrotic obstructive bowel lesion; intussusception (barium enema should be used with care to avoid perforation); rectal prolapse/chemotherapy; anorectal fistulas/abscess, surgical drainage and antibiotic therapy	Seen in 10–15% of some Middle Eastern populations; symptoms are those of focal granulomatous large bowel disease; may mimic chronic severe mucohemorrhagic diarrhea; residual fibrotic scarring may lead to permanent partial bowel obstruction or wall deformation.
Hepatosplenic disease	***S. mansoni*:** Praziquantel or oxamniquine; ultrasonography to define extent of disease ***S. japonicum*:** Praziquantel; ultrasonography	May be genetic predisposition to hepatosplenic syndrome; jaundice characteristically absent; liver cell function generally normal until terminal stages
	Hypersplenism: Therapy	Moderate and generally tolerable to patient
	Ascites: Low-salt diet and diuretics	End-stage disease can involve massive ascites; usually irreversible
	Bleeding esophageal varices: Esophagoscopy to confirm diagnosis (transfusion and esophageal sclerotherapy; surgical shunt for portal hypertension in severe cases)	Liver sonography can estimate degree of portal hypertension (measure distention of portal vein).
Urinary disease	**Uncomplicated:** Praziquantel (40 mg/kg) single dose; ultrasonography or intravenous pyelogram to detect silent obstructive disease	Most infections are mild and asymptomatic.
	Obstructive uropathy: Chemotherapy; may require surgery	Most lesions occur in lower third of ureters or bladder; pyelonephritis/Gram-negative sepsis common
	Urinary tract infection: Antibiotics plus antischistosomal therapy; pyelonephritis may require parenteral antibiotics	Primarily Gram-negative infections
	Renal failure: Hemodialysis or renal transplantation	Bilateral ureteral obstruction common; may be accompanied by *Salmonella* bacteriuria and/or bacteremia
	Bladder cancer: Radial surgery, chemotherapy usually only palliative	Usually lags 10–20 yr behind the peak *S. haematobium* infection; bladder calcifications may resolve over years if the patient remains schistosome free.
Cardiopulmonary disease	**Larval pneumonitis:** Self-limited course; may require short-course therapy with steroids if respiratory distress present	Occurs days to weeks after heavy cercarial exposure; symptoms last 2–4 wk (marked eosinophilia).
	Reactionary pneumonitis: Discontinue therapy until resolution; may require short-course therapy with steroids if respiratory distress is present	Loeffler-like syndrome seen during therapy of heavy infections.
	Cor pulmonale: Antischistosome therapy; symptomatic therapy	Heart affected after exposure to chronic abnormal pressure gradients; antischistosomal therapy prevents disease progression but does not lead to improvement.
Central nervous system disease	**Praziquantel therapy:** steroids recommended to reduce inflammation and size of space-occupying lesions	Most occur with *S. japonicum* (2–4% of cases); parietal lobe is the most common site.
	Cerebral: Focal, generalized seizures **Spinal:** Transverse myelitis of various degrees	Most occur with *S. mansoni*, also *S. haematobium*; lesions may be reversible.
Miscellaneous	***Salmonella*-schistosome syndrome:** Simultaneous antischistosomal and antibacterial therapy	Occurs most commonly in males 15–30 yr old; antibiotic response is dramatic; disease remains if schistosome disease is not also treated.
	Hepatitis B or C virus-schistosome disease: Antischistosomal therapy; transmission precautions for hepatitis B or C	Prognosis more guarded with dual infections

[a] Adapted from reference 46.

children; the greatest cercarial exposure usually occurs in boys aged 5 to 10 years. Older children may have less recreational exposure but are more likely to be exposed while performing chores such as agricultural activities, washing dishes and clothes, and bathing younger siblings. Although adults also have less recreational exposure to water, they also have partial acquired immunity.

Information on the presence of cercariae in water is important when only one of many snails is infected yet capable of shedding enough cercariae to maintain high endemicity. The use of sentinel mice for this purpose is inaccurate, time-consuming, and costly. Filtration is also limited and is associated with many problems. A PCR assay for the detection of *S. mansoni* cercariae in water has been developed. The high sensitivity of this test permits the detection of a single cercaria; a single cercaria could be detected in repeated tests of water filtrates (37).

Studies indicate that there was little transmission of *S. mansoni* in Puerto Rico during the first half of the 1990s and confirm physician anecdotal data that no new infections have been seen during the past few years (38). Recent trends indicate that human schistosomiasis is disappearing from Puerto Rico (39). The Puerto Rico Public Health Department reports that from 1990 through 26 September 2005, only 21 cases were reported. The last single case was in 2005 in a 69-year-old male from the east coast, this case probably being an "old" case. Although low transmission is still possible in Puerto Rico, data suggest that human schistosomiasis has been eliminated from this area. However, in other areas the news is not as good. The emergence of *S. mansoni* in Upper Egypt has not followed any particular pattern; however, the high prevalence detected in certain areas could be associated with the increasing abundance of the snail vector seen in the canals. Underreporting may also be associated with changing surveillance data. Water resource development projects involving the construction of artificial bodies of water and canals for irrigation have contributed to the extension of the disease. The migration of infected persons into virgin areas where suitable snail hosts reside also contributes to the spread of schistosomiasis. These two important factors make control programs difficult.

Schistosoma japonicum

Fujii in 1847 published the first clinical description of *S. japonicum* infections in his Katayama memoir. Katsurada in 1904 described the ova and adult worms that he detected in dogs, cats, and humans and named the parasite *S. japonicum*. Also known as the oriental blood fluke, the parasite is confined to the Far East and has been separated from *S. mekongi*, a more recently described species also found in the Far East. There is enough genetic variation in *S. japonicum* strains isolated from different areas of endemic infection that there may be separate species. *S. japonicum*, distributed in southern China, the Philippines, and parts of Indonesia, is the only schistosome species whose transmission is zoonotic. Over 40 species of wild and domesticated animals can serve as reservoir hosts for this parasite, including cows, pigs, dogs, cats, goats, sheep, and rats.

Life Cycle and Morphology

In addition to infections in humans, *S. japonicum* infections can be found in many mammals exposed to infected water; thus, there are many reservoir hosts, in contrast to other major human schistosomes (Table 20.1). Fully embryonated eggs without an operculum (70 to 100 μm by 50 to 65 μm) are passed in the feces. The eggs are smaller and somewhat more spherical than those of *S. haematobium* and *S. mansoni*. Near one end of the egg is a minute lateral spine; this may be absent in some strains (Fig. 20.15 and 20.16).

The adult worms resemble those of *S. haematobium* and *S. mansoni*; however, they lack integumentary tuberculations. *S. japonicum* adults are found in the radicles of the superior mesenteric vein draining the small intestine (Fig. 20.17). The female worm may contain as many as 50 eggs at one time in the uterus. Because of the higher egg production (3,000 eggs per worm pair per day) and the smaller egg size, an infection with just a few worm pairs can be very serious because the eggs, free in the general circulation, can be filtered out in the liver, the lungs, and even the CNS. The miracidium, released from the egg on contact with water, must find a suitable snail host (*Oncomelania* spp.) to continue the life cycle. Cercariae of *S. japonicum* tend to attach themselves to the water surface film, where they remain unless disturbed.

Clinical Disease

The pathology caused by *S. japonicum* is similar to that caused by *S. mansoni* infections and may include cercarial dermatitis as the first sequence in the disease process (Table 20.4). Although the severity is dependent on the worm burden, the disease has a poor prognosis because of the higher egg production capacity of *S. japonicum* females and the smaller eggs. *S. japonicum* infections have been associated with hepatocellular carcinoma and colorectal cancer. The mechanism of this schistosome-associated carcinogenic enhancement is unknown. The adult worms also have a greater tendency to be found in ectopic sites. Although adult worms have never been detected in the brain, cerebral schistosomiasis is a distinct syndrome that occurs with *S. japonicum* infections. The entire intestinal tract may be involved, but the large intestine is often the primary site and the disease is more severe there.

Acute Disease: Katayama Syndrome. Acute schistosomiasis (Katayama syndrome) is associated with heavy primary infec-

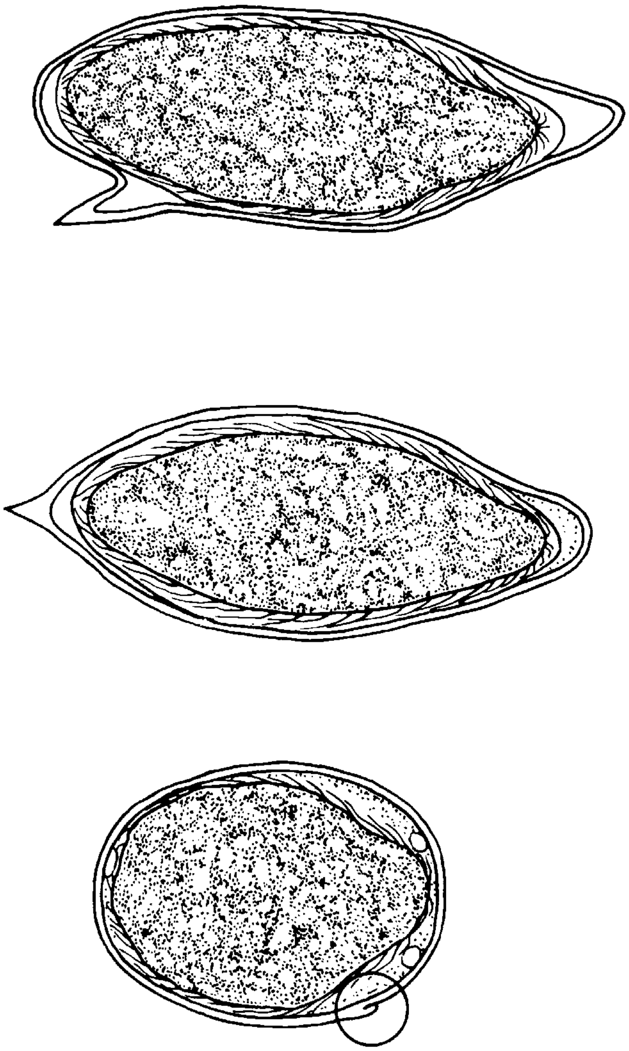


Figure 20.15 *Schistosoma mansoni* (top), *S. haematobium* (middle), and *S. japonicum* (bottom) eggs. Note how much smaller the *S. japonicum* egg normally is than those of the other two species, and note the small size of the lateral spine. (Illustration by Nobuko Kitamura.) doi:10.1128/9781555819002.ch20.f15

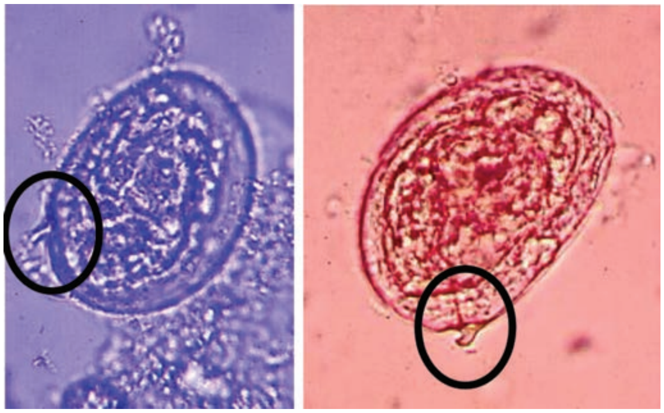


Figure 20.16 *Schistosoma japonicum* eggs. Note the small lateral spine within each oval. doi:10.1128/9781555819002.ch20.f16

tions and the initiation of egg production. The signs and symptoms include high fever, nocturnal fever peaks, coughing, generalized muscle pain, headache, hepatomegaly, lymphadenopathy, eosinophilia, and dysentery. Splenomegaly also occurs in approximately one-third of the patients, while all patients have eosinophilia. Diffuse pulmonary infiltrates are found; some patients have signs of meningoencephalitis. Generally, disease onset occurs about 40 or more days after exposure to a first infection or a large infection (40).

Intestinal Disease. As egg clusters accumulate, mucosal inflammation, hyperplasia, ulceration, microabscess formation, blood loss, and pseudopolyposis may occur. Lower abdominal pain is common and may be colicky and in the left lower quadrant. Diarrhea alternating with constipation is also common, with either visible blood, occult blood, or both. In severe chronic disease, bowel fibrosis and stenosis may occur with thickening of the mesentery.

Hepatosplenic Disease. Eggs that do not reach the intestinal lumen may be trapped or swept up by the portal blood flow. The eggs tend to accumulate in the venules within the portal triads, especially the left lobe; this is followed by granulomatous inflammation, fibrosis, venous obstruction, portal hypertension, and splenomegaly. Hepatosplenomegaly results from the granulomatous response surrounding the eggs. Although splenomegaly generally occurs during the early stages of chronic disease, the en-

Figure 20.17 *Schistosoma japonicum* paired adult male and female in a mesenteric vein. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch20.f17

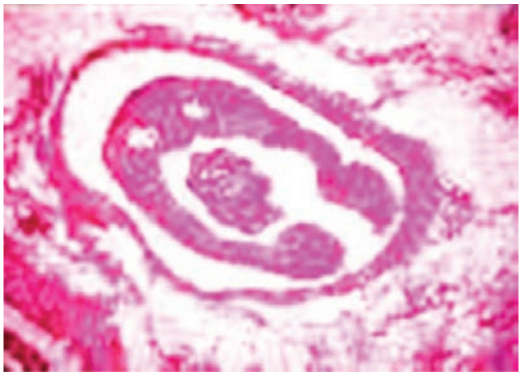


TABLE 20.4 Pathogenesis, pathology, and symptoms seen with *S. japonicum* infection

Stage of infection	Comments
Incubation (migration, maturation)	
Dermatitis	Associated with penetration of cercaria; urticarial rash; diarrhea at the end of this period
Lungs	Traumatic infiltrative changes, at times with hemorrhages, local accumulation of eosinophils, epithelioid cells, giant cells around pulmonary blood vessels (in wake of migrating larvae)
Liver	Acute hepatitis during larval growth in intrahepatic portal blood vessels
Small intestine	Hyperemia in wall of small intestine following arrival and during maturation of worms in superior mesenteric venules
Blood	Marked increase in number of circulating eosinophils
Egg deposition and extrusion	
Small intestine	Trauma with hemorrhage as eggs laid by female worms escape from venules through intestinal submucosa and mucosa into intestinal lumen; may be profuse dysentery; acute attack subsides in 3–10 wk; pseudotubercles form around eggs; repair occurs with scar tissue formation; giant cells develop within pseudotubercles, which become transformed to fibrous nodules
Blood	Blood picture changes from leukocytosis and hypereosinophilia to that of secondary anemia
Liver, spleen, lymph nodes	Liver increases in size as result of lesions around eggs; spleen becomes congested, fibrous reticulum increases, becomes greatly enlarged; mesenteric lymph nodes lose active lymphoid tissue
Tissue proliferation and repair	
Liver, spleen	Liver gradually decreases in size as parenchyma becomes replaced by scar tissue; spleen continues to enlarge; thoracic viscera are pushed upward; patient is weak with marked dyspnea on slight exertion; dilatation of superficial veins of abdomen and thorax is common
Intestine	Intestinal wall becomes thickened and fibrosed; papillomata develop on mucosa
Other problems	Ascites may be present; physical and mental retardation may occur in children; anemia is more pronounced
Blood vessels	Mesenteric and portal vessels become thrombosed; portal fibrosis due to egg deposition around the portal triads; severe fibrosis with white and hard tissue, frequently referred to as Symmer's fibrosis or pipe-stem fibrosis (fibro-obstructive hepatic schistosomiasis)
Cerebral disease	Patients classified as acute, chronic with granulomas or diffuse lesions, psychotic, or as having portal system encephalopathies; 2–4% (in some areas, this figure is higher) of all patients with *S. japonicum* schistosomiasis have acute or chronic cerebral involvement, including epileptic seizures

largement is due to tissue hyperplasia rather than granulomatous inflammation; thus schistosome eggs are rarely found in the spleen. In China, splenectomy is frequently used for severe hypersplenism (40).

After extended chronic disease, gross periportal fibrosis occurs with portal hypertension and secondary esophageal varices. Histology reveals wide bands of fibrous tissue extending along the portal tracts, hence the term "pipe-stem fibrosis," also seen with *S. mansoni* infections. These sequelae are more serious with *S. japonicum* infections, due primarily to the high daily egg production by the female worms. The parenchymal form of hepatic fibrosis occurs most commonly with *S. japonicum* infection and may be a subclinical find in groups who have been treated repeatedly and undergone overt liver disease resolution.

Central Nervous System Disease. Although not every patient with eggs in the CNS is symptomatic, symptoms can include Jacksonian convulsions and grand mal seizures. The use of praziquantel has led to improvement in over 75% of the cases in which it has been used. It is also unclear why Chinese schistosomiasis is associated with ectopic cerebral lesions while African disease tends to be associated with ectopic spinal lesions. Patients with CNS disease usually have the clinical manifestations of increased intracranial pressure associated with focal neurological signs; CNS disease is seen in from 2 to 4% of patients infected with *S. japonicum*. Apparently, the eggs reach the CNS through retrograde venous flow into the Batson vertebral epidural venous plexus, which connects the portal venous system and venae cavae to the spinal cord and cerebral veins. This route includes migration of the adult worms to sites close to the CNS followed by *in situ* deposition of eggs, or massive embolization of eggs from the portal mesenteric-pelvic system. The smaller, round eggs of *S. japonicum* reach the brain, while *S. mansoni* and *S. haematobium* eggs, which are larger and have sizable lateral and terminal spines, remain in the lower spinal cord (41).

Ectopic Disease. Egg granulomas of *S. japonicum* and, in rare cases, adult worms may be found outside the portal venous system, causing ectopic schistosomiasis. Many organs and tissues in a host's body can be involved, including the skin, subcutaneous tissue, lymph nodes, muscles, conjunctiva, tongue, brain, spinal cord, sympathetic nervous system, thyroid, breast, lung, cardiac muscle, pericardium, esophagus, ascitic fluid, kidney, ureter, urethra, bladder, adrenal cortex, inguinal hernia sac, and reproductive organs of both sexes (42).

Cancer and *S. japonicum* Infection. The incidence of colorectal cancer and *S. japonicum* infection correlates with the prevalence, intensity, and length of inflammatory bowel symptoms. This correlation has been reported from both China and Japan. In a study published in 2005 from rural China, schistosomiasis was thought to be responsible for 24% of colon cancers and 27% of liver cancers within that study group (43). Also, schistosome infection may have a negative effect on the prognosis of colorectal cancer. In a report by the National Cooperative Group on Pathology and Prognosis of Colorectal Cancer, the 5-year survival rate was 45.6% out of 430 cases complicated with schistosomiasis, which was significantly lower than in those without schistosomiasis (50.9% out of 2,717) (44).

Data regarding the association between schistosomiasis and colorectal cancer are highly suggestive; however, explanations for causes continue to be debated. Epithelial proliferation and polyp formation are associated with transformation to malignancy, and epithelial proliferation next to a chronic schistosomal lesion appears to be precancerous. Schistosome eggs as a potential causative agent for colorectal cancer may involve physical and chemical effects, changes of the secretion and excretion of the biliary acid, and the overall decline of host cellular immunity.

In reviewing the possible relationship between hepatic schistosomiasis and liver cancer, it appears that HBV and hepatitis C virus (known hepatic carcinogens) and related cirrhosis, as well as alcohol intake, may have a much stronger association than the actual *S. japonicum* infection (45).

Viral, Bacterial, and Alcohol Associations. Although hepatitis B has been reported to be more frequent in patients with schistosomiasis, the evidence is not that strong. Chronic bacteremic infections with *Salmonella* spp. have been associated with *S. haematobium*, *S. japonicum*, and *S. mansoni* infections (46). Common features of this association between *Salmonella* and *Schistosoma* infections include a long history of recurrent febrile illness, frequent isolation of *Salmonella* spp. from the blood, and chronic active schistosomiasis. The *Salmonella-Schistosoma* infection association is seen most frequently in males between the ages of 15 and 30 years. If the underlying schistosome infection is not treated, the *Salmonella* infection will continue to recur. However, this association appears to be rare for *S. japonicum* compared with the other schistosome species. There is evidence that there may be some association between alcoholic cirrhosis and hepatic fibrosis; however, details of specific mechanisms for this association are minimal.

Diagnosis

Schistosomiasis should be suspected in individuals who have had significant contact with water in areas of endemic infection. Symptoms of acute schistosomiasis include fever, diarrhea, abdominal pain, weight loss, and eosinophilia. Symptoms of chronic disease include chronic diarrhea, abdominal pain, and hepatomegaly or hepatosplenomegaly.

Diagnostic Procedures. Specific diagnosis of schistosomiasis by detection of eggs in stool specimens is possible only after egg production has begun. Eggs may be found in feces as early as 5 weeks after infection. The ease of egg detection depends on the worm burden and the duration of the infection. Patients with low worm burden or old (chronic) infections may have very few eggs in the feces or urine, and the infections are often not diagnosed because of the limits of sensitivity of examination techniques. Multiple stool examinations should be performed for any individual suspected of having schistosomiasis. Occasionally eggs of *S. japonicum* or *S. mansoni* are detected in the urine. The eggs of *S. japonicum* are more spherical than those of *S. mansoni* or *S. haematobium*; they measure 70 to 100 μm by 50 to 65 μm (Fig. 20.15 and 20.16). A minute lateral spine may be found at one end of the egg; however, it is absent in some strains of *S. japonicum*.

Data from China suggest that a single Kato-Katz smear may be quite adequate for detecting moderately to heavily infected individuals (100 eggs/g of stool); however, for estimating the true prevalence of infection within a community or for obtaining an accurate estimate of egg excretion, multiple Kato-Katz smears are recommended. It should be noted that large variations in egg counts and the lack of sensitivity of the Kato-Katz method, particularly in light and moderate infections, have been reported (47). Eggs also appear to be nonrandomly distributed in stool specimens between the center and surface of the stool; stirring the specimen prior to sampling decreased the count variability. Direct detection or concentration techniques can be used to detect eggs in stool. Direct microscopic examination of stool smears is not very sensitive but may be useful for screening purposes. The Kato thick smear is a simple and sensitive quantitation technique that has been used successfully in the field.

The zinc sulfate concentration technique is not recommended for schistosome eggs. The eggs will rupture and do not float but instead are found in the sediment. The formalin-ethyl acetate technique is recommended for concentrating eggs; however, because it involves fixation, it cannot be used to detect egg viability.

In chronic infections in which the worm burden is light, hatching tests can be performed. The miracidium hatching test has been widely used in China for many years; however, only 50 to 70% of the eggs will hatch, thus presenting problems for detection of light infections. The stool specimens are diluted with nonchlorinated water in a sedimentation flask or a beaker. The sides of the flask or beaker are covered with aluminum foil to prevent light from passing through. A light source is used to project a perpendicular light beam through the water at the top. Miracidia that hatch from the live eggs become concentrated in the light and can be detected swimming around. This motility can easily be observed with a hand lens. Aliquots of the surface water can be transferred to a small petri dish and observed under a dissecting microscope for miracidia. Observation periods should be frequent because of the limited life span of the miracidia. Ideally, observations should be made every 30 min over a period of 4 h. The hatching test is designed to mimic the conditions in nature with spring water and sunlight.

Biopsy Specimens. Although rectal biopsy specimens can be examined, this approach is neither practical nor convenient for population surveys. Also, it is difficult to differentiate dead from living eggs; these findings can lead to excessive therapy.

Sonography. Infection with *S. japonicum* currently affects about 1 million people, mainly in the Philippines and China. Individuals with chronic infection may develop liver fibrosis with portal hypertension, potentially resulting in life-threatening upper gastrointestinal hemorrhage. Apparently, there is no clear evidence about which sonographic features of infection with *S. japonicum* are related to portal hypertension and thus to a more likely fatal outcome. Using Doppler sonography, one study demonstrated that measurements of portal perfusion are a prognostic indicator of the risk of gastrointestinal hemorrhage and overall survival of these patients (48). This study also confirmed the suitability of Doppler sonography for research under tropical field conditions.

Antibody Tests. A large number of serologic tests have been used in the diagnosis of schistosomiasis (49); however, most have not been particularly useful because of cross-reactions with other helminth infections, continuation of elevated titers long after successful treatment, and slow immunologic response of the host. Because of the complex life cycle, a large number of antigens have been used in serologic tests. Only a few have been useful. The most frequently used tests are the circumoval precipitin test, the cercaria-Hullen reaction, the indirect fluorescent-antibody test, the indirect hemagglutination test, and ELISA (21, 49). See Algorithm 20.1.

ELISA methods using soluble adult worm or egg antigens for the detection of antibodies have been used as screening tests, and infection has then been confirmed by the EITB test (49). Individuals infected with schistosomes have elevated IgE and IgG4 responses, as in those with other helminthic infections. Few studies have investigated the persistence of antibodies over time after therapy (49). It is possible that high titers posttherapy reflect treatment failure and reinfection. In another study, the overall diagnostic effectiveness of the keyhole limpet hemocyanin dot-ELISA and the soluble egg antigen dot-ELISA for the detection of *S. japonicum* infection in regions of China with endemic infection was 97 and 92%, respectively (50).

ELISA has also been used to detect circulating schistosome antigens in the serum and urine of infected patients and may be the preferred method of diagnosis. In addition to diagnosis of infection, antigen levels have been used to assess disease severity in terms of intensity of infection and to monitor the efficacy of therapy. Individuals treated with praziquantel experience a rapid clearance of CAA and CCA; however, with other therapeutic drugs, clearance may take much longer.

Antigen Tests. The sensitivity of antigen tests is usually higher than that of routine fecal examinations for eggs; however, the specificity is lower. A rapid one-step enzyme immunoassay has been reported to have a sensitivity of 95% and a specificity of 100% (12). Unfortunately, antigen detection methods are not widely used in China. Limitations include the cost per case detected, complicated protocols, expensive reagents with a short shelf life, and a lack of complete understanding of the mechanics of circulating schistosome antigens. However, the potential advantages of the simple dipstick test(s) may lead to improved antigen testing, and the tests may become more widely available.

Molecular Tests. Differentiation between *S. japonicum* and *S. mekongi* eggs and/or cercariae via microscopic examination is difficult due to morphological similarities. A pyrosequencing assay designed to detect *S. japonicum* and *S. mekongi* DNA in fecal samples and infected snails was developed and evaluated as an alternative tool to diagnose schistosomiasis. The analytical sensitivity of the method enabled the identification of as little as a single cercaria artificially introduced into a pool of 10 noninfected snails and two eggs inoculated in 100 mg of noninfected fecal sample. These results suggest that the pyrosequencing assay is rapid, simple, sensitive, and accurate in identifying *S. japonicum* and *S. mekongi* in intermediate hosts and fecal samples of the final host (51). A recent study investigated iron localization in parasite eggshells. Iron is predominantly localized in pores in the eggshell. Magnetic microspheres spontaneously bound to parasite eggs without the presence of a magnetic field. *S. japonicum* eggs had a significantly higher affinity to bind microspheres than *S. mansoni* eggs. This magnetic fractionation method for isolation of parasite eggs from feces, which uses magnetic microspheres to form parasite egg-magnetic microsphere conjugates, lends itself to screening of large sample volumes and increased diagnostic sensitivity (52).

KEY POINTS—LABORATORY DIAGNOSIS

Schistosoma japonicum

1. Eggs cannot be detected in stool until the worms mature (may take 5 to 6 weeks after initial infection).
2. In very light or chronic infections, eggs may be very difficult to detect in stool; therefore, multiple stool examinations may be required. Biopsy and/or immunologic tests may be helpful in diagnosing infection in these patients.
3. The zinc sulfate flotation concentration method should not be used, since the eggs do not float; the sedimentation method is recommended.
4. It may be necessary to tap the coverslip to move the eggs; the small lateral spine may be difficult to see if debris is deposited on the egg (this is common).Also, remember that not all *S. japonicum* eggs will have the small lateral spine (no spine visible from any angle).
5. Occasionally, eggs of *S. japonicum* are detected in the urine.
6. Patients who have been treated should have follow-up ova and parasite examinations for up to 1 year to evaluate treatment.
7. In active infections, the eggs should contain live or mature miracidia. Examination for the confirmation of flame cell activity must be performed on fresh specimens; no preservatives can be used prior to this test or the hatching test.
8. During the hatching test, the light must not come too close to the surface of the water, since excess heat can kill the liberated larvae. Also, the water should be examined about every 30 min for up to 4 h.

Treatment

Praziquantel. Praziquantel is the drug of choice for treating infection with *S. japonicum*; the adult and pediatric doses are the same (60 mg/kg/day in three doses within 1 day). Side effects, which are usually mild and transient, include abdominal discomfort, dizziness, drowsiness, headache, fever, and loose stools. These effects usually disappear within 48 h. In heavily infected individuals, the side effects, although transient, may be very severe (51). Split doses have been recommended for treating heavy infections, but this may be a concern for population-based therapy, where individuals may not present themselves for follow-up therapy. Praziquantel has little effect on immature stages of the parasite, and this affects the cure rates as the immature parasites develop to maturity and egg production. The mechanism of action may involve glutathione *S*-transferase, a detoxifying enzyme found in many helminths. Cure rates have been reported to be from 70 to 95%. Praziquantel damages the tegument of the worm, thereby exposing the antigens to parasite-specific antibodies. The use of immunoglobulins along with praziquantel has been proposed to increase the efficacy of treatment.

Stem Cell Therapy. Stem cell therapy introduces new cells into damaged tissues, which help in treating many diseases and injuries. It has been documented that stem cell therapy is effective for the treatment of cancers, diabetes mellitus, Parkinson's disease, Huntington's disease, cardiovascular diseases, neurological disorders, and many other diseases. Recently, stem cell therapy has been introduced to treat parasitic infections. The culture supernatant of mesenchymal stem cells is found to inhibit activation and proliferation of macrophages induced by the soluble egg antigen of *S. japonicum*, and this treatment relieves *S. japonicum*-induced liver injury and fibrosis in mouse models (53). Although in the beginning stages, within the next few years stem cell therapy may become a therapeutic option for parasitic diseases.

Epidemiology and Prevention

S. japonicum infections occur in the Far East, including China, Indonesia, Japan, and the Philippines. There is a focus of enzootic infection in Taiwan; however, there is no evidence of locally acquired human infections. In addition to humans, numerous animals (cats, cattle, dogs, goats, horses, sheep, pigs, mice, rats, and water buffalo) are naturally infected in many areas of endemic infection. The infection is transmitted by *Oncomelania* snails, which are operculated, amphibious, and able to survive prolonged periods of desiccation. On contact with water, the snails become active again and shed infective cercariae. The widespread use of night soil as fertilizer significantly increases the occupational hazards of the disease for rice farmers. There is evidence that congenital *S. japonicum* infection of pigs can occur if sows are infected during mid to late pregnancy; this may have implications not only for pigs but also for other mammalian hosts of schistosomes, including humans. Praziquantel tends to be highly effective against *S. japonicum* in pigs without causing pathologic side effects in the liver.

The geographic distribution of the disease depends on the distribution of the intermediate snail hosts and the opportunity to infect humans and snails. Schistosomiasis is transmitted from infected people defecating or urinating in or near water where the appropriate snail host resides. Infections can persist indefinitely in humans. Most infected individuals have low worm burdens, but a few have very heavy infections. It is the latter group that probably makes the greatest contribution to the dissemination of schistosomiasis. The highest rates of infection have been found in children; the greatest cercarial exposure usually occurs in boys aged 5 to 10 years. Older children may have less recreational exposure but are more likely to be exposed while performing chores such as agricultural activities, washing dishes and clothes, and bathing younger siblings. Although adults also have less recreational exposure to water, they also have partial acquired immunity.

S. japonicum is a major public health concern in the People's Republic of China, with about 800,000 people infected and another 50 million living in areas at risk of infection. It is predicted that, long-term, the Three Gorges Dam will have an impact on the transmission of schistosomiasis in the geographic region of Dongting Lake, located in Hunan Province in southern China. Asian schistosomiasis has been endemic in this region for centuries, with a significant impact on the public health of the local population. Even with dedicated control programs, the snail habitats are huge (1,768 km^2) and are increasing at a rate of 34.7 km^2 annually. With the completion of the Three Gorges Dam, it was anticipated that this project would substantially increase the range of the snail habitats and hence increase the number of new cases of schistosomiasis with a high risk of transmission. However, based on a recent study, it appears that there has been no immediate impact of the dam on schistosomiasis transmission downstream (54). In many areas, human reinfections after praziquantel therapy remain very high (up to 20%), primarily due to occupational water contact. Efforts aimed at control or eradication of the infection in the future will remain very complex (55).

Domestic cattle and buffalo are the most important reservoir hosts in China, with humans and animals frequently coming in contact at water sources, which lead to increased transmission. Although praziquantel is used for periodic treatment of bovines, the animals are prone to reinfection.

Vaccine Development. While decades of vaccine development have included the discovery and testing of several candidate antigens, none have shown consistent and acceptable high levels of protection. Selection and manufacture of the final product will be difficult, particularly regarding funding issues related to Good Manufacturing Practice scale-up for extensive veterinary coverage and to support any future human trials. The final product will probably be a mixture of several molecules and will be used in an integrated program of control that would also include health education and targeted chemotherapy (56). A number of immunogens have been identified which could be used for vaccines, and vaccine development is ongoing (56–58). A number of other initiatives are also under way and include newer drugs, improved diagnostics, disease risk prediction, and transmission control using satellite-based remote sensing.

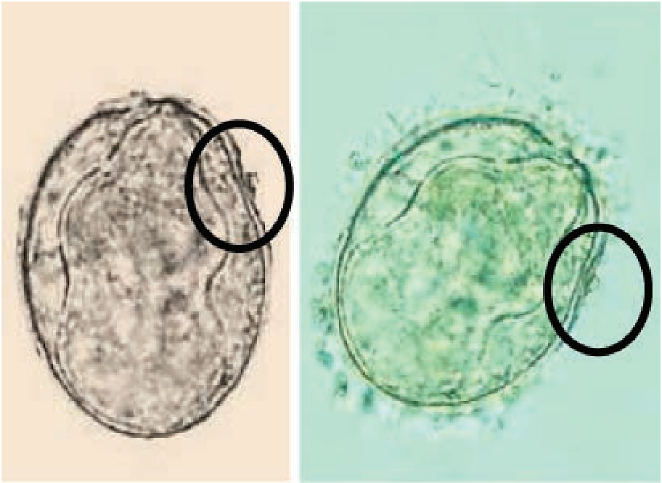
Schistosoma mekongi

Schistosomiasis resembling the disease caused by *S. japonicum* has been known to exist in the lower Mekong River basin since 1957. Further investigation has shown the etiologic agent to be a new species, *S. mekongi*; this species is responsible for most of the cases reported from mainland Indochina and has a prevalence of up to 68% in some areas (59).

Life Cycle and Morphology

Dogs as well as humans may serve as definitive hosts (Table 20.1). The adult worms are found in the mesenteric veins and resemble those of *S. japonicum*, although they lack tuberculations. Egg production occurs within 4 to 6 weeks after infection, and adult females may have many eggs in the uterus. The eggs of *S. mekongi* are similar in shape to those of *S. japonicum* but are smaller. They are subspherical, with a size range of 30 to 55 µm by 50 to 65 µm, and they have a small lateral spine near one end (Fig. 20.18). Fully embryonated eggs without an operculum are passed in the feces. When the egg comes in contact with freshwater, the miracidium is released. The miracidium must infect the aquatic snail *Lithoglyphopsis aperta* to continue the life cycle.

Figure 20.18 *Schistosoma mekongi* eggs. Note the subspherical shape and smaller size than *S. japonicum* eggs. Also note the very small lateral spine (ovals). (Courtesy of CDC Public Health Image Library.) doi:10.1128/9781555819002.ch20.f18



Clinical Disease

Few clinical studies have been conducted in areas of endemic infection; however, the pathologic effects of *S. mekongi* infections appear similar to those of other human intestinal schistosomes. *S. mekongi* appears to be no more or less pathogenic than *S. japonicum*, with hepatosplenomegaly and portal venous hypertension being the most important serious sequelae (Table 20.5). Brain involvement of the infection has also been confirmed (60).

TABLE 20.5 Distribution of infection and morbidity due to *Schistosoma mekongi* in northern Cambodia

Information	Comments
Study population 1	Household surveys in villages on east and west banks of Mekong River (1,396 participants)
Diagnostic method	Single stool examination (Kato-Katz smear)
Overall infection prevalence	49.3%
Infection intensity	118.2 eggs/g of stool
Prevalence and intensity distribution	No differences between east and west shores of Mekong (see below)
Morbidity	Severe morbidity common; hepatomegaly (48.7%), splenomegaly (26.8%), visible diverted circulation (7.2%), ascites (0.1%); significantly more hepatomegaly, splenomegaly, diverted circulation seen on west bank
Prevalence details	West bank, 71.8%; east bank, 71.9%; age group most affected was 10–14 yr; west bank, 172.4 eggs/g; east bank, 194.2 eggs/g
Peak age group (10–14 yr)	West bank, hepatomegaly (88.1%); east bank, hepatomegaly (82.8%)
Study population 2	20 primary schools on east and west banks (2,391 participants, age 6–16 yr)
Diagnostic method	Single stool examination (Kato-Katz smear)
Overall infection prevalence	40.0% (range 7.7–72.9% per school)
Infection intensity	110.1 eggs/g of stool (range 26.7–187.5 eggs/g per school)
Prevalence and intensity distribution	Significantly higher in schools on the east bank
Morbidity	Severe morbidity common; hepatomegaly (55.2%), splenomegaly (23.6%), visible diverted circulation (4.1%), ascites (0.5%), reported blood (26.7%) and mucus (24.3%) in stool; significantly more hepatomegaly, splenomegaly, diverted circulation, and blood in stool in schools on the east bank
Prevalence details	No differences in sex for prevalence and intensity of infection or prevalence of hepatomegaly; boys more likely to have splenomegaly, ascites, and bloody stools; overall prevalence and intensity of infection highly associated in this study population

Diagnosis

Schistosomiasis should be suspected in individuals who have had significant contact with water in areas of endemic infection, particularly along the Mekong River in Laos and Cambodia. Symptoms of acute schistosomiasis include fever, diarrhea, abdominal pain, weight loss, and eosinophilia. Symptoms of chronic disease include chronic diarrhea, abdominal pain, and hepatomegaly or hepatosplenomegaly.

Diagnostic Procedures. Specific diagnosis of schistosomiasis by detection of eggs in stool specimens is possible only after egg production has begun. Eggs may be found in feces as early as 5 weeks after infection. The ease of egg detection depends on the worm burden and the duration of the infection. Patients with low worm burden or old (chronic) infections may have very few eggs in the feces or urine, and the infections are often not diagnosed because of the limits of sensitivity of examination techniques. Multiple stool examinations should be performed for any individual suspected of having schistosomiasis. The eggs of *S. mekongi* are more round and tend to be smaller than those of *S. japonicum*; they measure 50 to 65 µm by 30 to 55 µm (Fig. 20.18). A minute lateral spine is found at one end of the egg; however, it may be very difficult to see.

Direct detection or concentration techniques can be used to detect eggs in stool. Direct microscopic examination of stool smears is not very sensitive but may be useful for screening purposes. The Kato or Kato-Katz thick smears are simple and sensitive quantitation techniques that have been used successfully; however, sensitivity is lacking in light infections.

The zinc sulfate concentration technique is not recommended for schistosome eggs. The eggs rupture and do not float but instead are found in the sediment. The formalin-ethyl acetate technique is recommended for concentrating eggs; however, because it involves fixation, it cannot be used to detect egg viability.

For diagnosis of chronic infections in which the worm burden is light, hatching tests can be performed. See chapter 4 and the discussion under *S. mansoni*.

Antibody Tests. A large number of serologic tests have been used in the diagnosis of schistosomiasis (19); however, most have not been particularly useful because of cross-reactions with other helminth infections, continuation of elevated titers long after successful treatment, and slow immunologic response of the host. Because of the complex life cycle, a large number of antigens have been used in serologic tests. Only a few have been useful. The most frequently used tests are the circumoval precipitin test, the cercaria-Hullen reaction, the indirect fluorescent-antibody test, the indirect hemagglutination test, and ELISA (19, 21). See Algorithm 20.1.

ELISA methods using soluble adult worm or egg antigens for the detection of antibodies have been used as screening tests, and infection has been confirmed by the EITB test (21). Individuals infected with schistosomes have elevated IgE and IgG4 responses, as in those with other helminthic infections. Few studies have investigated the persistence of antibodies over time after therapy (21). It is possible that high titers posttherapy reflect treatment failure and reinfection.

The dipstick dye immunoassay was developed in China for the detection of antibodies against *S. japonicum*. This assay has also been tested in the diagnosis of *S. mekongi* infections in Cambodia and Laos. When testing Cambodians, the sensitivity was 97.1%; when testing patients from Laos, the sensitivity was 98.6%. Cross-reactivity was found in patients infected with *Opisthorchis viverrini*; interpretation may therefore be more difficult in areas where both *S. mekongi* and *O. viverrini* are endemic (61).

Antigen Tests. ELISA has also been used to detect circulating schistosome antigens in the serum and urine of infected patients and may be the preferred method of diagnosis (20). In addition to diagnosis of infection, antigen levels have been used to assess disease severity in terms of intensity of infection and to monitor the efficacy of therapy (20). Individuals treated with praziquantel experienced a rapid clearance of CAA and CCA; however, with other therapeutic drugs, clearance may take much longer.

KEY POINTS—LABORATORY DIAGNOSIS

Schistosoma mekongi

1. Eggs cannot be detected in stool until the worms mature (may take 4 to 6 weeks after initial infection).
2. In very light or chronic infections, eggs may be very difficult to detect in stool; therefore, multiple stool examinations may be required. Biopsy and/or immunologic tests may be helpful in diagnosing infection in these patients.
3. The zinc sulfate flotation concentration method should not be used; the sedimentation method is recommended.
4. It may be necessary to tap the coverslip to move the eggs; the small lateral spine may be difficult to see if debris is deposited on the egg (this is common) (Fig. 20.18).
5. These eggs tend to be more round and are smaller than those of *S. japonicum*.
6. Patients who have been treated should have follow-up ova and parasite examinations for up to 1 year to evaluate treatment.
7. In active infections, the eggs should contain live or mature miracidia. Examination for the confirmation of flame cell activity must be performed on fresh specimens by using the wet mount or hatching test; no preservatives can be used prior to the wet mount examination or the hatching test.
8. During the hatching test, the light must not come too close to the surface of the water, since excess heat can kill the liberated larvae. Also, the water should be examined about every 30 min for up to 4 h.

Treatment

Praziquantel is the drug of choice for treating infection with *S. mekongi*; the adult and pediatric dose is the same (60 mg/kg/day in three doses within 1 day). Side effects may include abdominal discomfort, dizziness, drowsiness, headache, fever, and loose stools; however, these effects usually disappear within 48 h. In heavily infected individuals, the side effects may be more severe. Split doses have been recommended for treating heavy infections, but this may be a concern for population-based therapy, where individuals may not present themselves for follow-up therapy. Praziquantel has little effect on immature stages of the parasite. Cure rates have been reported to be from 70 to 95%. Praziquantel damages the tegument of the worm, exposing the antigens to parasite-specific antibodies. In treating the patient with brain involvement mentioned above, the response to praziquantel was excellent but repeated courses of corticosteroid therapy were required to suppress recurring neurologic symptoms (60).

Epidemiology and Prevention

S. mekongi infections occur in the Mekong River basin in Cambodia, Laos, and Thailand. Dogs and rodents also harbor the infection, which presents a problem for prevention of the spread of infection. The snail *Lithoglyphopsis aperta*, a member of the family *Hydrobiidae*, is aquatic, lives on solid debris in water, has a limited distribution, and is responsible for transmission of the disease. Although the snail host is sensitive to common molluscicides, control measures have not been undertaken on a concerted basis.

A baseline epidemiologic survey of *S. mekongi* was conducted along the Mekong River in northern Cambodia; this comprehensive study documents for the first time the public health importance of schistosomiasis mekongi in this area and the high level of morbidity associated with the infection (Table 20.5) (62). Schistosomiasis control in both Laos and Cambodia was based on universal treatment campaigns and resulted in a dramatic decrease of the prevalence of the infection and in morbidity control. However, even if the disease and the infection have been satisfactorily controlled, transmission still occurs, and in very limited areas the prevalence reaches rates of more than 15%. Today, 1.5 million people are estimated to be at risk of infection in Laos and in Cambodia.

Schistosoma malayensis

S. malayensis is a member of the *S. japonicum* complex, is closely related to *S. mekongi*, and differs genetically from the latter by ≈10%. In Asia, *S. japonicum*, *S. mekongi*, and *S. malayensis* cause human infection, with *S. japonicum* being the most dangerous. *S. malayensis* eggs from naturally and experimentally infected hosts are most similar to those of *S. mekongi*, with eggs of both species being, in general, smaller than those of *S. japonicum*. The egg index for *S. malayensis* is usually higher than for *S. japonicum* and lower than for *S. mekongi*. Apparently, among a stable population at risk of *S. malayensis* the prevalence is low. These findings support the hypothesis that *S. malayensis* is a zoonotic infection in humans and is unlikely to become a significant public health problem.

Schistosoma haematobium

Urinary schistosomiasis was common in the Nile River valley and has been found during examination of the kidneys of Twentieth Dynasty Egyptian mummies. Bilharz in 1851 documented the disease when he recovered adult worms from the veins of autopsy cadavers. Leiper in 1915 definitively proved the existence of two separate species, *S. haematobium* and *S. mansoni*, which until that time had been confused with one another. Although schistosomiasis is rare in western Europe, it is important to consider infectious disease in patients with obstructive uropathy, particularly in the context of traveling in or immigration from areas where *S. haematobium* infection is endemic.

Life Cycle and Morphology

Humans are the only significant reservoir hosts of *S. haematobium* (Table 20.1). Fully embryonated eggs without an operculum (112 to 170 µm by 40 to 70 µm) escape from the body in the urine. The eggs are light yellowish brown and contain a conspicuous terminal spine (Fig. 20.15 and 20.19).

The adult male worm contains minute integumentary tuberculations, smaller than those found on adult *S. mansoni* males. *S. haematobium* adults reside in the vesical and pelvic plexuses of the venous circulation. The adult female can contain 20 to 100 eggs in the uterus at one time. In addition to the vesical and pelvic plexuses, oviposition may occur in the rectal venules. To maintain the life cycle, the miracidium must find a suitable snail host (*Bulinus* sp.) when it is released from the egg in freshwater. The immature larval worms, after reaching the liver, may migrate via the inferior mesenteric veins to the rectal vein in order to mature. However, they most commonly migrate through the hemorrhoidal and pudendal veins to the vesical and pelvic plexuses. Within 3 months of exposure to cercariae, egg production begins.

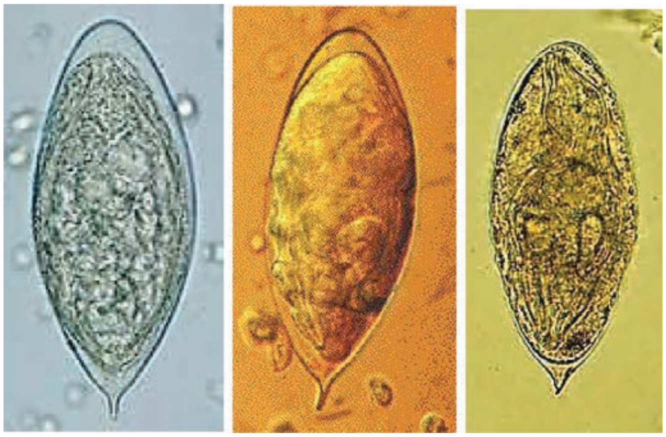


Figure 20.19 *Schistosoma haematobium* eggs. Note the terminal spine and slight size variations.
doi:10.1128/9781555819002.ch20.f19

Clinical Disease

Urogenital Disease. Light infections with urinary schistosomiasis usually produce no symptoms; however, in early disease there may be dysuria and hematuria due to cystitis from deposited eggs. Depending on the worm burden, symptoms may include dysuria, frequency, and terminal or total hematuria. Hematuria is so common that in some areas of endemic infection this phenomenon was considered to be analogous to menarche in girls (63). Symptoms are usually not seen for 3 to 6 months and may take a year or more to develop. Physical examination is usually normal, but urinalysis may reveal many red blood cells and a few white blood cells on microscopic examination; reagent strip results may indicate hematuria and proteinuria. Chronic disease may lead to major problems, including obstructive uropathy, chronic bacteriuria, bladder carcinoma, and bladder calcification (Table 20.6).

Eggs are most highly concentrated in the tissues of the bladder and lower ureter (Fig. 20.20 and 20.21). As the eggs become trapped in the tissues, granulomas and pseudoabscesses form, leading to fibrosis and ulceration. With extensive fibrosis, the bladder loses its contractility. The urethra frequently is occluded because of hyperplasia, polyp formation, and discharge of purulent debris plugs from the bladder. The ureters are also frequently involved, and obstruction can cause urine reflux, hydronephrosis, retrograde infections, and renal failure. Heavy infections in males may involve the penis, resulting in elephantiasis due to blockage of the scrotal lymphatics by egg deposition. Detection of *S. haematobium* eggs in 43% of semen samples with increased levels of eosinophil cationic protein suggests that the male genital organs are frequently affected (64).

Up to 75% of women with urinary schistosomiasis have *S. haematobium* eggs in the genitalia; urinary schisto-

TABLE 20.6 Disease manifestations seen with *Schistosoma haematobium* infection

Disease manifestation	Comments
Obstructive uropathy	Hydronephrosis, hydroureter, pyelonephritis, renal failure often silent clinically but can be demonstrated by intravenous pyelogram, ultrasonography, or renogram function testing; most lesions occur in lower third of ureters or bladder.
Pyelonephritis	Secondary to obstruction; Gram-negative sepsis; may be the leading cause of mortality in urinary disease.
Renal failure	Bilateral ureteral obstruction common; may be the direct result of obstruction, pyelonephritis, or ureterovesicular urinary reflux.
Chronic bacteriuria	Infection rates up to 5% in schoolboys with *S. haematobium* infection; coliforms are responsible, with *Salmonella* being the most common in some studies; treatment usually unsuccessful unless schistosome infection is also treated and schistosomal mucosal lesions are eliminated.
Bladder carcinoma	Bladder irritation, gross hematuria, weight loss, metastasis (inguinal, femoral, and retroperitoneal lymph nodes) tend to lag 10–20 yr behind age at which *S. haematobium* infection peaks; most cases diagnosed in patients aged 40–49 yr; biopsy is the usual method of confirmation; urinary cytology may be helpful.
Bladder calcification	The "fetal head" sign is seen in abdominal X rays and is caused by calcium deposits around schistosome eggs in the bladder and uterine walls (Fig. 17.20); sonography can also be used to see calcification in the bladder walls; although bladder loses contractility due to fibrotic walls, it does not become rigid; calcification resolves over years if patient remains free of schistosomiasis.
Cutaneous disease	Has been previously reported but is an unusual presentation; skin lesions, appearing 3 yr after exposure, have been seen as the only manifestation of schistosomiasis; routine biopsy confirmed infection; awareness of this infection when evaluating patients with unusual skin lesions who have traveled in areas of endemicity is recommended (100).
Other body sites	Myelopathy (acute transverse myelitis and subacute myeloradiculopathy) of the lumbosacral region is the most common neurological manifestation of *S. mansoni* or *S. haematobium* infection, whereas acute encephalitis of the cortex, subcortical white matter, basal ganglia or internal capsule is typical of *S. japonicum* infection (101).

somiasis is associated with sandy patches in the lower genital tract. Eggs have also been detected in biopsy specimens from vaginal mucosa. Female genital schistosomiasis (FGS) is often associated with eggs in the cervix, vagina, and/or vulva, as seen during examination of a wet cervical biopsy specimen crushed between two glass slides. There is significant correlation between the size of the genital lesions and the number of eggs per square millimeter of crushed tissue. Women with FGS also tend to have more tumors in the vulva than do women with schistosomiasis limited to the urinary tract (65). In one study, only 43% of the patients with FGS had hematuria. Also, since FGS frequently exists in women with scanty or no egg excretion in the urine and since this disease represents both an individual and public health hazard in areas of endemic infection, mass treatment targeted to women of childbearing age should be a consideration (66). *S. haematobium* eggs have been associated with homogeneous yellow sandy patches, mucosal bleeding, and abnormal blood vessels. However, the finding of eggs was not a predictor for ulcers, papillomata, leukoplakia, polyps, or cell atypia (65). Genital pathology due to sequestered *S. haematobium* ova is partially reversible 2 to 9 weeks after the adult worms have been killed by praziquantel. A reduction in inflammatory responses can be detected in histologic sections and vaginal lavage fluid. However, these sandy patch lesions associated with contact bleeding and vessel abnormalities may be refractive to treatment for up to 12 months and may be an important risk factor for potential acquisition and transmission of HIV (65, 67).

Obstructive Uropathy. Egg deposits in the ureter walls are the general causes of obstructed urinary flow; conditions leading to obstruction include polypoid patches, fibrosis, ureteritis cystica, calculi, and the total number of eggs present. Often these patients remain asymptomatic other than having symptoms related to cystitis or pain from the ureters.

Bladder Calcification. Calcified eggs produce the typical image, which consists of confluent sandy patches as seen in Fig. 20.22 (left). The tissues of the bladder are not calcified and may continue to function normally; however, the visual radiologic picture suggests a heavy infection. Calcified eggs can also be seen in the ureters and seminal vesicles and less often in the colon.

Bladder Carcinoma. Carcinoma of the bladder has been frequently noted in patients infected with *S. haematobium*. Many factors have been suggested as agents promoting schistosome-associated bladder cancer (Table 20.7). *N*-Nitroso compounds in association with secondary bacterial infections of the urinary tract may contribute to the high prevalence of bladder cancer (68). Bladder cancer is the most prevalent cancer in Egyptians. Many of the tumors involve the posterior wall of the bladder and are

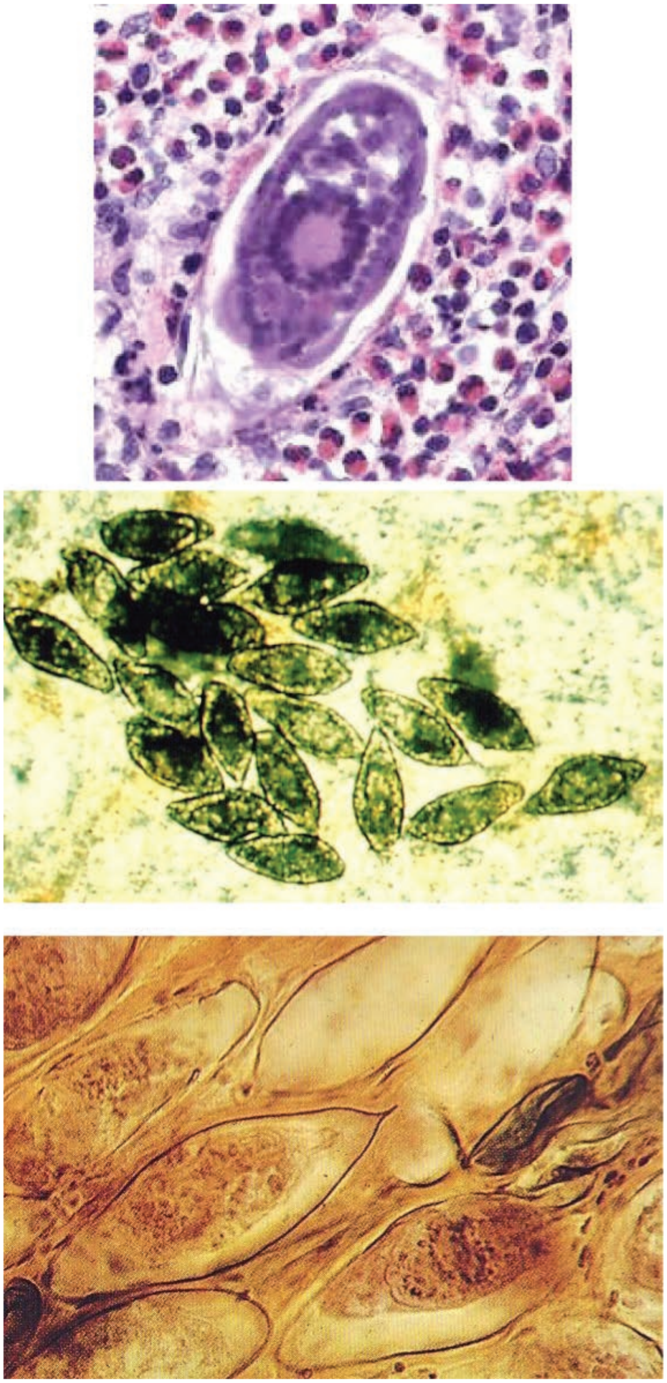


Figure 20.20 *Schistosoma haematobium*. (Top) Egg in a bladder biopsy (hematoxylin and eosin stain; courtesy of the CDC Public Health Image Library). (Middle) *S. haematobium* eggs in bladder biopsy; note the terminal spine. (Bottom) Eggs in a bladder biopsy specimen (higher magnification). (Middle and bottom images from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch20.f20

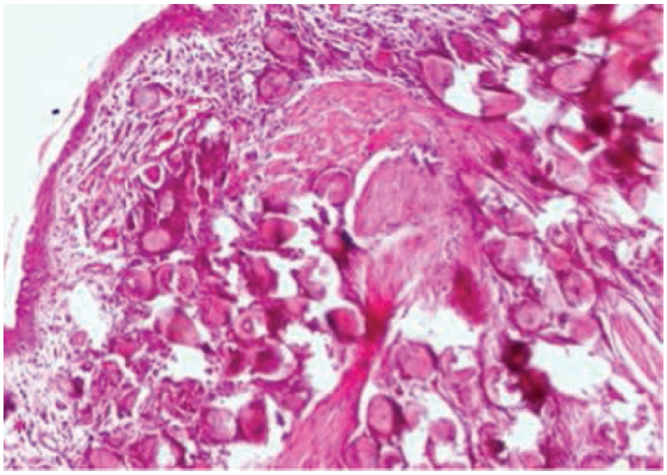


Figure 20.21 Calcified *Schistosoma haematobium* eggs in a ureter embedded in the submucosa and muscle (hematoxylin and eosin stain). (Courtesy of http://ispub.com/IJID/7/1/12041.)
doi:10.1128/9781555819002.ch20.f21

noted to occur more frequently in males than in females. The extent of *S. haematobium* infection plays a significant role in the induction of different types of carcinoma, since squamous cell carcinoma is usually associated with moderate and/or high worm burdens while transitional cell carcinoma occurs more frequently in areas associated with lighter parasite loads. The predominance of squamous cell carcinoma in urinary bladder tissues in patients with schistosomiasis is probably due to continuous exposure to the larger quantities of carcinogens (*N*-nitroso compounds) in urine in patients with the disease (69).

Another factor that may play a major role in bladder carcinogenesis is continuous physical irritation and inflammation produced by *Schistosoma* eggs in the bladder mucosa. The adult *S. haematobium* worms inhabit the veins of the perivesical plexus, where the female lays eggs. Some eggs pass through the bladder mucosa and are excreted in the urine, while other eggs are trapped in the tissue. A chronic inflammatory response occurs, with the invasion of histiocytes and other inflammatory cells into the bladder, the formation of granulomas, and eventually fibrosis. Also, egg penetration through the mucosa stimulates urothelial hyperplasia and cell turnover. Generally in schistosomiasis patients, cancer originates throughout the bladder but rarely in the trigone, a frequent site of tumor origin in non-*Schistosoma* cases. Following a major decline in the prevalence of urinary schistosomiasis in Egypt, the occurrence of transitional cell carcinoma of the bladder has replaced that of squamous cell carcinoma. This change in the pattern of infection suggests the importance of *Schistosoma*-associated bladder cancer that, elsewhere, may be more widespread than is presently thought (70).

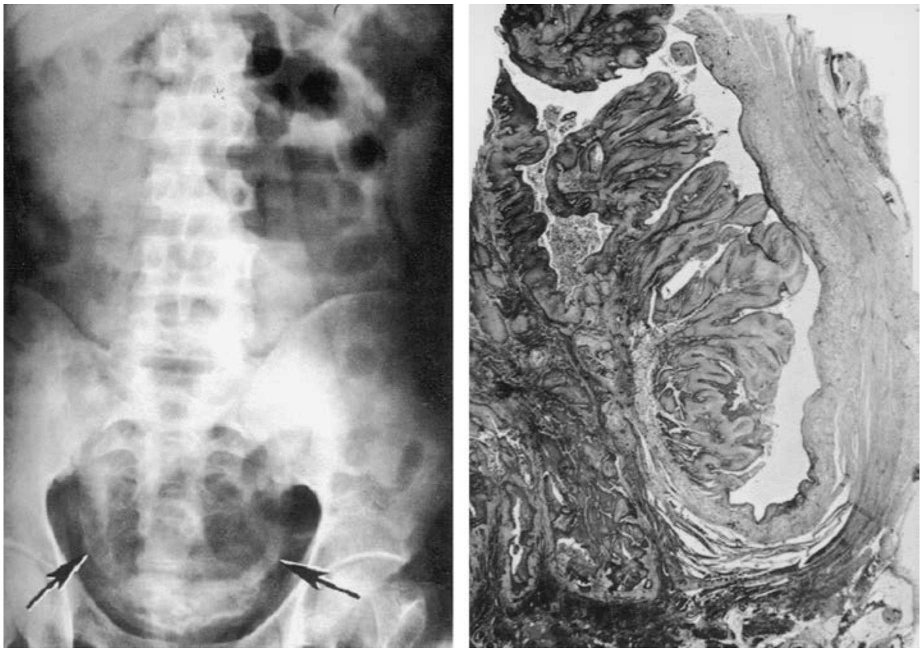


Figure 20.22 *Schistosoma haematobium*. (Left) X ray of the pelvis in a 24-year-old Egyptian with schistosomal obstructive uropathy. Linear calcification is seen in the base of the bladder and along the lower ureters, which are dilated (arrows). (Armed Forces Institute of Pathology photograph, courtesy of Marcia Young.) (Right) Urinary bladder. An exophytic squamous cell carcinoma is present in the central portion of the photograph, and infiltrating squamous cell cancer extends to the left. Magnification, ×4. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch20.f22

Other Body Sites. Periportal fibrosis with hepatomegaly and splenomegaly has been noted in patients in areas where infections are endemic. Splenic enlargement has been correlated with the intensity of *S. haematobium* infection. Cardiopulmonary disease can develop in patients with hepatosplenic schistosomiasis; eggs are carried via the mesenteric veins to the lungs through systemic collateral veins. Schistosomal cor pulmonale tends to be rare in infections with *S. haematobium*; eggs can be found in lung tissue at autopsy but are generally not clinically relevant. Although any *Schistosoma* species may involve the CNS, *S. mansoni* and *S. haematobium* more commonly involve the spinal cord than the brain. Severe disease presents as transverse myelitis.

As with *S. mansoni*, chronic bacteremic infections with *Salmonella* are seen, particularly in cases of chronic active schistosomiasis. Also, schistosomal appendicitis appears to be specific to infection with *S. haematobium* and is an uncommon cause of appendicitis in areas where schistosomiasis is not endemic. Therapy requires antischistosomal medication in addition to surgery (71).

Diagnosis

Diagnostic Procedures. *S. haematobium* eggs are usually detected in the urine, although in heavy infections they may also be found in the stool. The terminal hematuria portion of the urine specimen may contain numerous eggs trapped in the mucus and pus (Fig. 20.23). Peak egg excretion occurs between noon and 3 p.m. Samples collected during this time, or during a 24-h urine collection without preservatives, may be used for examination. Urine can be examined under a microscope after sedimentation or centrifugation. It is important to use saline and not water for the concentration procedures; this will avoid premature hatching of the eggs. Nuclepore filtration is an excellent method for the concentration of eggs in urine. Some data indicate that egg output in urine is an accurate method of confirming the diagnosis and shows less day-to-day variation than in ELISA detection of schistosome circulating antigens in urine (72).

Use of reagent strips to detect hematuria and proteinuria in areas of endemic infection has proven to be an effective screening technique for *S. haematobium* infections (73). The assay correctly identified 87% of egg-negative controls and 98% of parasitologically confirmed cases, including those in six individuals who had been treated but not cured. Also, antigen was detected in the urine of three individuals from whom two specimens had to be examined microscopically to confirm the infection. This suggests that the test is more sensitive than microscopy. To increase the sensitivity of the reagent strip tests, the tests should be performed during the period of peak egg excretion in the urine (noon to 3 p.m.). Although the use of reagent strips allows one to rapidly screen a population at risk, other conditions can yield a positive result. The strips should be used in conjunction with microscopic examination or serologic testing for confirmation of infection. Reagent strips coated with monoclonal antibodies (MAbs) to CCA were developed for testing urine specimens for *S. haematobium*. The levels of CCA are much higher in the urine than are the levels of CAA. Although not as sensitive as ELISA methods, this technique is suitable for screening large populations in areas of endemic infection. Eosinophilia has also been used to diagnose infections, with a positive predictive value of 80%.

TABLE 20.7 Mechanisms of bladder carcinogenesis due to *Schistosoma haematobium*

Mechanism	Comments
Inflammatory cells (IC)	Macrophages and neutrophils are important sources of endogenous oxygen radicals that are implicated in the formation of carcinogenic *N*-nitrosamines; IC induce mutations, sister chromatid exchanges, and DNA strand breaks; increased numbers of IC may enhance carcinogenic potential of aromatic amines by increasing their rate of activation.
Microorganisms	Hospitalized patients (39–66%) had bacteriuria; interaction between schistosomiasis and bacterial infection appears to depend on gender (more common in males than females); there may be a symbiotic relationship between worms and bacteria; bacterial infection of the urinary tract increases the risk of bladder cancer in patients with problems other than schistosomiasis; individuals with bacterial cystitis and schistosomiasis are potentially more exposed to nitrate and/or nitrite, increasing the risk of *in situ* formation of *N*-nitrosamines.
Genetic changes	Specific genes associated with neoplastic progression in schistosomal bladder cancer include the activation of H-*ras* (the *ras* gene family consists of frequently activated oncogenes), inactivation of *p53*, and inactivation of the retinoblastoma gene; alterations in genes and/or proteins can change function, leading to uncontrolled cell growth and tumor formation. Mutation of the coding sequence of the *p53* tumor suppressor gene is the most frequent genetic alteration in a number of human malignancies; 6 of 7 Egyptian bladder cancer patients had *p53* mutations; the frequency of *p53* mutations varies with different grades of schistosomiasis-associated bladder cancer; alterations in the *p53* gene may modulate gene expression for regulation of relevant DNA repair processes, as well as cell division and death by apoptosis. *Bcl-2* is overexpressed in some schistosomiasis-associated bladder cancers (expressed at high levels in squamous cell carcinoma but not transitional cell carcinoma); may be upregulated in later stages of tumor progression; *Bcl-2* may also be related to tumor cell lineage. A 9p gene, possibly *CDKN2*, may contribute to the development of the majority of schistosomiasis-associated bladder tumors, but the genes on 9q play a much less important role (102). Detection of early genetic changes may be valuable in identifying high-risk individuals.
Diet	Dietary fat is consistently high on a list of associated factors (attenuated when other diet components are included); small amounts of *N*-nitroso compounds are found in Egyptian food (cheese, fava beans, raw salted fish), all of which are consumed almost daily.
Carcinogen metabolism	Increased levels of enzymes responsible for activation of carcinogenic *N*-nitroso compounds, aromatic amines, and polycyclic aromatic hydrocarbons have been found in schistosomiasis; may increase and prolong bladder exposure to DNA-damaging agents; nitrite levels are about 20 times higher in *S. haematobium*-infected patients with bladder cancer than in those without bladder cancer; these compounds may play a role not only in initiation of the carcinogenic process, but also in its progression.
Carcinogens, consequences of DNA damage	Damage to bladder tissue DNA may be responsible for initiation of bladder cancer.

Antibody Detection. A recognized obstacle to serodiagnosis of acute or recent schistosome infection is the prolonged seronegative period. Antibody production in newly infected individuals usually starts 4 to 7 weeks after infection. However, although the majority of patients seroconvert within 3 months, prolonged seronegative periods of up to 6 months have been described. A large number of serologic tests have been used in the diagnosis of schistosomiasis (49); however, many have not been particularly useful because of cross-reactions with other helminth infections, continuation of elevated titers long after successful treatment, and slow immunologic response of the host. Because of the complex life cycle, a large number of antigens have been used in serologic tests. Only a few have been useful. The most frequently used tests are the circumoval precipitin test, the cercaria-Hullen reaction, the indirect fluorescent-antibody test, the indirect hemagglutination test, and ELISA (49, 74). See Algorithm 20.1.

ELISA methods using soluble adult worm or egg antigens for the detection of antibodies have been used as screening tests, and infection has then been confirmed by the EITB test (49). Individuals infected with schistosomes have elevated IgE and IgG4 responses, as in those with other helminthic infections. Few studies have investigated the persistence of antibodies over time after therapy (49). It is possible that high titers posttherapy reflect treatment failure and reinfection.

Antigen Tests. Based on 92% specificity and 90% sensitivity, an ELISA using MAb 290-2E6-A is one of the most promising assays for immunodiagnosis of *S. haematobium* infections (75). Another study of children from Kenya indicates that the soluble egg antigen (SEA) ELISA compared well with microhematuria within egg count classes and with egg counts within hematuria classes (76). Levels of SEA and egg output have shown similar correlations with sonographically detectable pathology; these correlations were better than

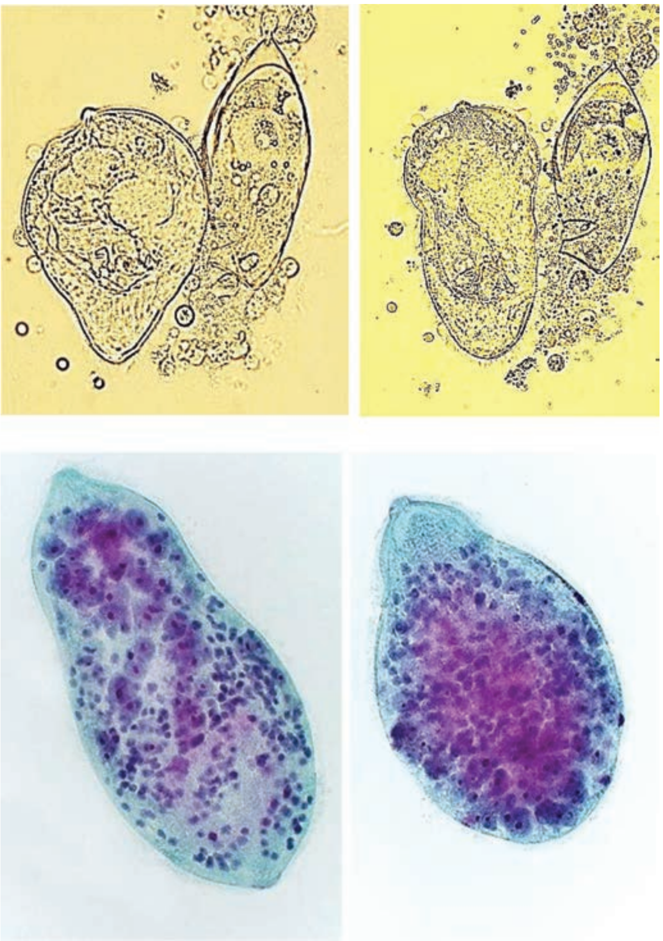


Figure 20.23 *Schistosoma haematobium*. (Upper images) Urine sediment from patient from the Sudan; note the miracidium larvae that have emerged from the eggshells (courtesy of Betty A. Forbes, Medical College of Virginia). (Lower images) Stained miracidium larvae (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman, from E. K. Markell). doi:10.1128/9781555819002.ch20.f23

the correlation between hematuria and pathology (76). Another antigen capture ELISA using MAb 128C3/3/21 has shown a correlation between the level of circulating antigen and the intensity of infection as measured by egg counts. There appears to be a direct relationship between antigen level and disease severity, as monitored by sonography. Also, a purified recombinant serine protease inhibitor that is species specific for *S. haematobium* has been used in serologic assays.

Using the SEA MAb, circulating schistosome antigen was undetectable in serum from patients with other parasitic infections but was detected in 90.5% of serum samples and 94% of urine samples from *S. haematobium*-infected patients. A significant reduction in circulating schistosome antigen levels was detected in serum and urine samples 12 weeks after praziquantel therapy (77). No cross-reactivity with *S. mansoni* was seen; circulating schistosome antigen was undetectable in 94% of serum samples and 84% of urine samples from *S. mansoni*-infected patients.

A MAb-based dipstick assay has been used with a sensitivity of 98.8% and specificity of 53.6%. Although the detection of microhematuria and proteinuria had a higher specificity, the sensitivity was much lower than that of the dipstick assay (78). The use of a circulating cathodic antigen strip approach for the diagnosis of urinary schistosomiasis has also been developed for pre- and posttreatment results with a sensitivity of 88% and 96%, respectively (79).

Molecular Tests. Recent studies using an internal transcribed spacer-based real-time PCR for the detection of *Schistosoma* DNA in urine samples have shown high sensitivity and specificity based on controls and known microscopy-positive samples (80). In field control situations, there is a need to detect low-level infections even in children, where infection occurs in the absence of eggs or hematuria. Unfortunately, these cases can still infect snails and perpetuate the cycle. In both situations a simple urine specimen, filtered through a filter paper, can be used for DNA detection, and a PCR procedure will give a definitive result (81).

KEY POINTS—LABORATORY DIAGNOSIS

Schistosoma haematobium

1. Eggs cannot be detected in urine until the worms mature (may take up to 3 months after initial infection).
2. In very light or chronic infections, eggs may be very difficult to detect in urine; therefore, multiple urine examinations may be required. Biopsy and/or immunologic tests may be helpful in diagnosing infection in these patients.
3. Both 24-h and spot urine samples should be examined as wet mounts (after concentration using no preservatives [use saline so hatching will not occur]); the urine specimens should be collected with no preservatives. These eggs are also occasionally recovered in stool, and so both urine and stool specimens should be examined.
4. The membrane filtration technique using Nuclepore filters can be very helpful in diagnosing infection with *S. haematobium*.
5. Patients who have been treated should have follow-up ova and parasite examinations for up to 1 year to evaluate treatment.
6. In active infections, the eggs should contain live or mature miracidia. Examination for the confirmation of flame cell activity must be performed on fresh specimens using the wet mount or hatching test; no preservatives can be used prior to the wet mount or the hatching test. If the specimens are concentrated, use saline rather than water to prevent premature hatching of the eggs.

(continued)

KEY POINTS—LABORATORY DIAGNOSIS

***Schistosoma haematobium* (continued)**

7. During the hatching test, the light must not come too close to the surface of the water, since excess heat can kill the liberated larvae. Also, the water should be examined about every 30 min for up to 4 h.
8. It is important to remember that the small and less commonly seen miracidium larva of *S. haematobium* may be present in the urine; motility in unpreserved specimens or stained morphology could confirm this diagnosis.
9. It is also possible to see *S. haematobium* eggs in semen specimens, even when repeated urinary and fecal examinations and serologic tests are negative.

Treatment

Praziquantel is the drug of choice for treating infections with *S. haematobium*. It can be taken orally in a single dose and is well tolerated by the host. Side effects, which are usually mild and transient, include abdominal discomfort, dizziness, drowsiness, headache, fever, and loose stools. These effects usually disappear within 48 h. In heavily infected individuals, the side effects, although transient, may be very severe. Split doses have been recommended for treating heavy infections, but this may be a concern for population-based therapy, where individuals may not present themselves for follow-up therapy. Data suggest that a single dose of praziquantel (40 mg/kg of body weight) may have a longer-lasting effect than was previously thought: possibly more than 2.5 years. In certain population-based treatment programs, it appears that a 20-mg/kg dose of praziquantel may be sufficient to provide control of morbidity due to urinary schistosomiasis (82). Praziquantel has little effect on immature stages of the parasite, and this will affect the cure rates as the immature parasites develop to maturity and egg production. The mechanism of action may involve glutathione *S*-transferase, a detoxifying enzyme found in many helminths. Cure rates have been reported to be 70 to 95%. Praziquantel damages the tegument of the worm, thereby exposing the antigens to parasite-specific antibodies. Adults are more resistant to schistosome infection than are children, and the switch to an "adult" response suggests that praziquantel treatment may have an immunizing effect, with benefits beyond a transient reduction in infection levels (83). Treatment can significantly reduce urinary tract morbidity despite reinfection; important risk factors in adulthood are cumulative intensity and duration of infection during early adolescence. Data also indicate that therapy given in childhood or adolescence decreases the risk for some but not all manifestations of *S. haematobium* infection in later adult life (84).

Although the exact mechanism by which praziquantel kills the parasite is not well understood, it is known that the immune response of the host plays an important role in drug efficacy. It is possible that disease states of humans that lead to immunodeficiencies, such as infection with HIV-1, may render praziquantel less effective in treating schistosomiasis. However, recent studies indicate that persons with HIV-1 infection can be treated effectively for schistosomiasis with praziquantel (67).

Therapeutic failure of praziquantel in the treatment of *S. haematobium* infection in Brazilians returning from Africa has been reported. Patients were treated with a single dose of praziquantel (40 mg/kg, single dose). Two additional treatments were given, and cystoscopy between 6 and 24 months after each treatment confirmed the presence of viable eggs. These findings confirm the possible need for repeated therapy, as well as using the urinary bladder biopsy as the criterion of cure (85).

The efficacy of praziquantel during the incubation period of schistosomiasis has recently been studied for the first time (86). In a group of 18 tourists infected with *S. haematobium* in Mali, the authors observed the efficacy of praziquantel given at different phases of the infection. Early praziquantel treatment from days 10 to 15 after exposure was less effective than later treatment (days 28 to 40) in preventing acute schistosomiasis, while neither treatment effectively prevented chronic schistosomiasis.

Patients with *S. haematobium* infection are suitable recipients for kidney transplantation, although they are at risk for urologic complications. Long-term urologic follow-up is recommended, including urethrocystoscopy, due to the increased risk for bladder cancer.

Metrifonate, an organophosphorus cholinesterase inhibitor, is an alternative to praziquantel for the treatment of *S. haematobium* infections. The drug is given orally over a number of weeks. Side effects, which are mild and transient, include abdominal discomfort, diarrhea, dizziness, headache, vomiting, and weakness. Metrifonate should not be given to patients who have been recently exposed to insecticides or who are using therapeutic agents that can potentiate cholinesterase inhibition. It has been noted that the dorsal tegumental surface of adult male schistosomes contains acetylcholinesterase and nicotinic acetylcholinesterase receptors, which may be the target of metrifonate. Because the acetylcholine receptor expression is increased when the worms pair and become sexually mature, it appears that acetylcholine plays a vital function in nutrient transport. Because metrifonate requires multiple administrations, it is less convenient to use in community-based control programs.

Epidemiology and Prevention

S. haematobium infections occur in Africa, Asia Minor, Cyprus, the islands off the African east coast, and southern Portugal; there is a focus of endemic infection in India.

Humans appear to be the only important reservoir hosts, although naturally infected monkeys, baboons, and chimpanzees have been found. The intermediate snail host, *Bulinus* sp., can survive in the mud when the water dries up. The snails retain their infectivity and resume shedding cercariae when the rainy season begins. In some areas, cercariae are found in areas where infected snails are absent, and sometimes cercariae are absent in the presence of infected snails.

Data suggest that fewer than 1 in 100 contacts result in infection and less than 1 in 1,000 result in egg output. This suggests that there may be substantial attrition of invading cercariae even in naive individuals (87).

In many areas of endemic infection, awareness of urinary schistosomiasis and its symptom of blood in the urine is high, but specific knowledge about the parasite, its vector, and the interaction between them in the life cycle is lacking. Activities that require behavior and attitude modification can be identified and encouraged as components in the control of schistosomiasis.

An *S. haematobium*-derived recombinant glutathione *S*-transferase (Sh28GST) vaccine is capable of significantly reducing worm fecundity in experimentally infected primates. Although Freund's complete adjuvant induced higher levels of protection, the efficacy of *Mycobacterium bovis* as an adjuvant appeared sufficient to justify consideration of its use in a vaccine against human urogenital schistosomiasis (88).

A summary of the control methods that can be used for all types of schistosomiasis is given in Table 20.8. All of these initiatives are coupled with public health communication and training for populations in areas of endemic infection.

Schistosoma intercalatum

S. intercalatum infections were first noted by Fisher in the early 1930s, and the species has been extensively described. The infection has been found in central and western Africa. In areas where both *S. haematobium* and *S. intercalatum* are endemic, hybridization with the two trematodes appears to be occurring; natural hybrids have been found in Loum, Cameroon, and natural hybridization has been suspected in Gabon. Also, it appears that the competitive exclusion between *S. mansoni* and *S. intercalatum* may be an important factor restricting the distribution of *S. intercalatum* in Africa. *S. intercalatum* causes an intestinal form of schistosomiasis similar to that caused by *S. mansoni* but characterized by lesions, mainly situated at the rectum and sigmoid level. Urinary schistosomiasis caused by *S. haematobium* has a tendency to supplant rectosigmoidal schistosomiasis caused by *S. intercalatum*, especially in endemic areas where hybridization between the two species of schistosomes is occurring.

Life Cycle and Morphology

The life cycle is similar to that of the other schistosomes. Adult worms are found in the mesenteric veins, and eggs are shed in the feces. Eggs are fully embryonated without an operculum and resemble *S. haematobium* eggs in shape, having a terminal spine; however, *S. intercalatum* eggs tend

TABLE 20.8 Schistosomiasis control measures

Approach	Comments
Chemotherapy	
Mass	Most appropriate in population with prevalence rates of >30%; all members of population treated (no attempt at individual diagnosis
Targeted population	Relies on identification of individual moderate to heavy egg excretors; follow-up for evaluation of cure
Snail control	
Molluscicide use	Niclosamide commonly used; well suited for arid areas (seasonal infections) and small habitats; unfortunately, snail populations can reestablish themselves within 3 mo of molluscicide application
Biologic control	Use of natural predators of snail hosts (ducks, fish, turtles, fungi, other snails); not realistic on a mass level
Environmental modification	Prevention and/or reduction of breeding; site modification to produce detrimental conditions; cementing or closing over irrigation ditches; increasing canal water flow
Reduction of water contact and contamination	
Domestic water supplies	Clean water availability; however, population may continue to use contaminated water sources
Sanitary excreta disposal	Mandatory; combined with health education on biological transmission and importance of control measures; improvement in living standards is important
Vaccination	Continued problem of reinfection is a problem due to slow maturation of natural resistance; several antigens have reached an advanced stage of development and are undergoing testing; vaccination will be used in conjunction with all other control methods; vaccines can be used to attenuate infection and/or to prevent pathology and/or to reduce worm burdens

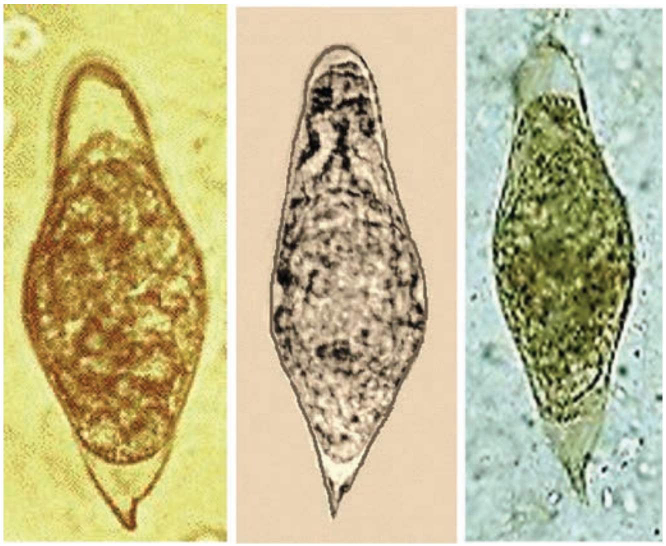


Figure 20.24 *Schistosoma intercalatum* eggs. The miracidium tends to have a bulge in the middle, the eggs are usually larger than *S. haematobium*, and they are seen in stool rather than urine. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
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to have a bulge in the middle (Table 20.1; Fig. 20.24). The eggs are 140 to 240 μm by 50 to 85 μm in size and can be differentiated from *S. haematobium* eggs since they are Ziehl-Neelsen acid-fast positive. This technique cannot be totally relied on, because if hybridization occurred between *S. intercalatum* and *S. haematobium*, the eggs identified from the urine by DNA analysis would be Ziehl-Neelsen stain positive. The snail intermediate hosts are *Bulinus forskalii, B. globosus*, and *B. africanus*.

Clinical Disease

Clinical manifestations are similar to those of *S. mansoni* infections. Pathology is confined to the mesenteric-portal system; rectal bleeding appears to be the most common finding. Rare pulmonary infections have also been noted. Other symptoms can include pain in the left iliac fossa with tenesmus, anorexia, nausea, abdominal pain, and diarrhea. There may also be changes in liver function tests. The prevalence and intensity of infection were noted to increase during the first 2 decades of life and gradually decrease thereafter. As with other species of *Schistosoma*, there is an association between *Salmonella* bacteremia and infection with *S. intercalatum*. Without treatment for schistosomiasis, the *Salmonella* infection cannot be cured. Complications of *S. intercalatum* infection include severe rectal or genital lesions.

Diagnosis

The infection can be diagnosed by finding the characteristic eggs in feces or in squash preparations of tissues from the rectum; occasionally, eggs are also found in the urine. The miracidium often has a bulge in the middle.

KEY POINTS—LABORATORY DIAGNOSIS

Schistosoma intercalatum

1. Eggs cannot be detected in stool until the worms mature (may take from 4 to 6 weeks after initial infection).
2. In very light or chronic infections, eggs may be very difficult to detect in stool; therefore, multiple stool examinations may be required. Biopsy and/or immunology may be helpful in these patients.
3. The zinc sulfate flotation concentration method should not be used; the sedimentation method is recommended.
4. It may be necessary to tap the coverslip to move the eggs; the terminal spine may be difficult to see if debris is covering the egg (this may occur).
5. Occasionally eggs of *S. intercalatum* are detected in the urine.
6. Patients who have been treated should have follow-up ova and parasite examinations for up to 1 year to evaluate treatment.
7. In active infections, the eggs should contain live or mature miracidia. Examination for the confirmation of flame cell activity must be performed on fresh specimens, using a wet mount or the hatching test; no preservatives can be used prior to the wet mount examination or the hatching test.
8. During the hatching test, the light must not come too close to the surface of the water, since excess heat can kill the liberated larvae. Also, the water should be examined about every 30 min for up to 4 h.

Treatment

Praziquantel is the drug of choice for treating infections with *S. intercalatum*. It can be taken orally in a single dose and is well tolerated by the host. Side effects, which are usually mild and transient, include abdominal discomfort, dizziness, drowsiness, headache, fever, and loose stools. These effects usually disappear within 48 h. A single dose of praziquantel (40 mg/kg of body weight) has been recommended.

Epidemiology and Prevention

S. intercalatum infections occur in the same geographic areas as *S. mansoni* infections, i.e., western and central Africa (Cameroon, Equatorial Guinea, Gabon, Nigeria, Democratic Republic of Congo, and Sao Tome), Egypt, Madagascar, and the Arabian peninsula. Human infections are nearly all derived from human sources, even though nonhuman primates, insectivores, marsupials, and rodents can carry the infection. Disease spread is linked to seasonal migration of nomad populations, as well as migrant African laborers. In regions of endemic infection, prevalences of 5 to 25% have been found. The infection is transmitted by *Bulinus* sp., an aquatic snail.

Review of 333 reports related to imported cases of schistosomiasis in Europe showed that male patients accounted for 64% of all cases. The average age of all patients was 29.5 years. The majority of patients were of European origin (53%). Europeans traveled predominantly for tourism (52%). Travel for people from areas of endemic infection was related to immigration and refuge (51%) and visits to relatives and friends (28%). The majority of infections were acquired in Africa; 92 infections were clearly attributable to *S. haematobium*, 130 to *S. mansoni*, and 4 to *S. intercalatum*. Praziquantel was the only treatment used, and no deaths were recorded (89).

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Intestinal Protozoa: Amebae

Entamoeba histolytica
Entamoeba dispar
Entamoeba moshkovskii
Entamoeba bangladeshi
Entamoeba hartmanni
Entamoeba coli
Entamoeba polecki
Entamoeba gingivalis
Endolimax nana
Iodamoeba bütschlii
Blastocystis spp.

Entamoeba histolytica

Entamoeba histolytica was first described by Losch after it was isolated in Russia from a patient with dysenteric stools. Although Losch found the organisms in human colonic ulcers at autopsy and was able to induce dysentery in a dog that was inoculated rectally with the patient's stools, he failed to recognize the causal relationship. This organism was eventually more fully investigated and differentiated from *Entamoeba coli* and *Entamoeba hartmanni* with respect to both morphology and pathogenesis (1). Based on the genome, this organism has a number of metabolic adaptations shared with two other amitochondrial protist pathogens, *Giardia lamblia* and *Trichomonas vaginalis*. These adaptations include the use of oxidative stress enzymes usually associated with anaerobic prokaryotes, as well as reduction or elimination of most mitochondrial metabolic pathways. There is evidence for lateral gene transfer of bacterial genes into the *E. histolytica* genome, a large number of novel receptor kinases, and expansions of a variety of gene families, including those associated with virulence (2).

E. histolytica has been recovered worldwide and is more prevalent in the tropics and subtropics than in cooler climates. However, infection rates in unsanitary conditions in temperate and colder climates have been found to equal those seen in the tropics. The World Health Organization reported that *E. histolytica* causes approximately 50 million cases and 110,000 deaths annually (3). Only malaria and schistosomiasis surpass amebiasis as leading parasitic causes of death.

At least 11 amebae are found in the mouth or intestinal lumen (*E. histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba bangladeshi*, *E. hartmanni*, *E. coli*, *Entamoeba polecki*, *Entamoeba gingivalis*, *Endolimax nana*, *Iodamoeba bütschlii*, and *Blastocystis* spp.). Of these, only *E. histolytica* and *Blastocystis* spp. have been considered to be pathogenic; however, *E. moshkovskii* and *E. bangladeshi* have been implicated as causing disease in humans, particularly in infants (4).

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E. histolytica versus *E. dispar*

Although a large number of people throughout the world are infected, only a small percentage will develop clinical symptoms. Morbidity and mortality due to *E. histolytica* vary, depending on the geographic area, the organism strain, and the patient's immune status. For many years, the issue of pathogenicity has been very controversial, with essentially two points of view. Some thought that what was called *E. histolytica* was really two separate species of *Entamoeba*, one being pathogenic and causing invasive disease and the other being nonpathogenic and causing mild or asymptomatic infections. Others thought that all organisms designated *E. histolytica* were potentially pathogenic, with symptoms depending on the result of host or environmental factors, including the intestinal flora.

In 1961, with the development of successful axenic culture methods requiring no bacterial coculture, sufficient organisms could be obtained for additional studies. Approximately 15 years later, reports indicated that *E. histolytica* clinical isolates could be classified into groups by using starch gel electrophoresis and review of banding patterns related to specific isoenzymes (5). The four isoenzymes are glucophosphate isomerase, phosphoglucomutase, malate dehydrogenase, and hexokinase. Sargeaunt concluded from this work that there are pathogenic and nonpathogenic strains (zymodemes) of *E. histolytica* that can be differentiated by isoenzyme analysis. On the basis of analysis of thousands of clinical isolates, he also concluded that the zymodeme patterns were probably genetic rather than phenotypic.

Others argued that the bacterial flora present in the gut played a role in the potential pathogenicity of *E. histolytica*. Some of the *in vitro* culture studies indicated that bacteria enhance the virulence of *E. histolytica*. During the 1980s and early 1990s, these issues were discussed, with a number of questions still remaining unanswered (6).

According to Diamond and Clark (7), the most logical explanation of their failure to obtain conversion of "nonpathogenic" *E. histolytica* isolates to the "pathogenic" form during axenization of the amebae was culture contamination. In reviewing this possibility, they used a method based on analysis of stable DNA polymorphisms that allows the identification of individual pathogenic isolates. The DNA patterns obtained with the "converted" amebae were identical to those of reference isolates present in the laboratory at the time of conversion (7). Diamond and Clark also found that transfer of very few pathogenic organisms was necessary for the pathogens to become established in a nonpathogenic culture. Within the context of these studies, cross-contamination fully explained the conversion, and the designation of specific pathogenic *E. histolytica* and nonpathogenic *E. dispar* was confirmed (7).

Studies confirming the differences between the two organisms include direct sequencing of the PCR-amplified small-subunit rRNA gene of *E. dispar* and the design of primers for rapid differentiation from *E. histolytica*; differences in the *E. histolytica* and *E. dispar* phosphoglucomutases; molecular biology of the differences in the hexokinase isoenzyme; differences in the secretion of acid phosphatase; and the detection of DNA sequences unique to *E. histolytica* (8).

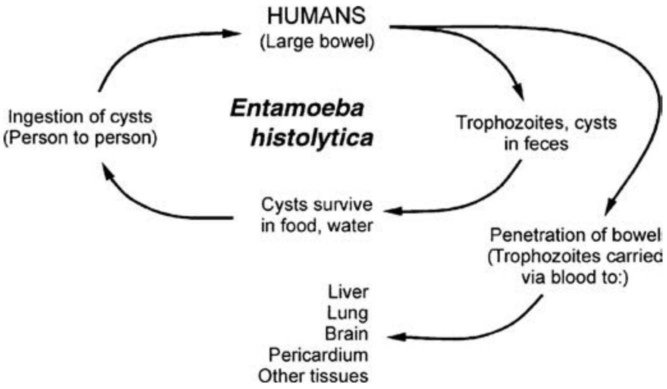
Genetic differences may not totally explain the overall differences. Among 79 *E. histolytica* isolates obtained from different geographic regions, a total of 53 different genotypes were observed among 63 isolates. The most extensive variations among the four loci were found in the serine-rich *E. histolytica* protein (SREHP) locus. However, no association between clinical presentation and the SREHP genotype was demonstrated (9).

Research has centered on explaining the molecular differences between the pathogenic *E. histolytica* and the nonpathogenic *E. dispar*. However, the molecules considered the most important for host tissue destruction (amebapore, galactose/*N*-acetylgalactosamine inhibitable lectin, and cysteine proteases) can be found in both organisms. Pathogenicity differences may be related to the composition and properties of the surface coat components (or pathogen-associated molecular patterns) and the ability of the innate immune response to recognize these components, thus eliminating the organisms (10). Targets of the host immune system modulation appear to be both neutrophils and macrophages, which are unable to abort the infection even when present at the site of the lesion (11). The total body of evidence supports differentiation of the pathogenic *E. histolytica* from the nonpathogenic *E. dispar* and their classification as two distinct species (12).

Life Cycle and Morphology

The life cycle is shown in Fig. 21.1. The cyst form is the infective form for humans.

Figure 21.1 Life cycle of *Entamoeba histolytica*.
doi:10.1128/9781555819002.ch21.f1



Morphology of Trophozoites (Table 21.1). Living trophozoites vary in size from about 12 to 60 μm in diameter. Organisms recovered from diarrheic or dysenteric stools are generally larger than those in a formed stool from an asymptomatic individual (Fig. 21.2 and 21.3). The motility has been described as rapid and unidirectional, with pseudopods forming quickly in response to the conditions around the organism. The motility may appear to be sporadic. Although this characteristic motility is often described, it is rare to diagnose amebiasis on the basis of motility seen in a direct mount. The cytoplasm is differentiated into a clear outer ectoplasm and a more granular inner endoplasm.

When the organism is examined on a permanent stained smear (trichrome or iron hematoxylin), the morphological characteristics of *E. histolytica*/*E. dispar* are readily seen. The nucleus is characterized by having evenly arranged chromatin on the nuclear membrane and having a small, compact, centrally located karyosome. The cytoplasm is usually described as finely granular with few ingested bacteria or debris in vacuoles. In organisms isolated from a patient with dysentery, red blood cells (RBCs) may be visible in the cytoplasm, and this feature is diagnostic for *E. histolytica*. Most often, infection with *E. histolytica*/*E. dispar* is diagnosed on the basis of organism morphology without the presence of RBCs.

Morphology of Cysts (Table 21.2). For reasons that are not specifically known, the trophozoites may condense into a round mass (precyst) and a thin wall is secreted around the immature cyst. There may be two types of inclusions within this immature cyst, a glycogen mass and highly refractile chromatoidal bars with smooth, rounded edges. As the cysts mature (metacyst), there is nuclear division with the production of four nuclei; on rare occasions, eight nuclei are produced and the cysts range in size from 10 to 20 μm. Often, as the cyst matures, the glycogen completely disappears; the chromatoidals may also be absent in the mature cyst. Cyst morphology does not differentiate between *E. histolytica* and *E. dispar*. Cyst formation occurs only within the intestinal tract; once the stool has left the body, cyst formation does not occur. The one-, two-, and four-nucleated cysts are infective and represent the mode of transmission from one host to another (Fig. 21.1 to 21.3).

Infection. After cyst ingestion, no changes occur in an acidic environment; however, once the pH becomes neutral or slightly alkaline, the encysted organism becomes active, with the outcome being four separate trophozoites (small, metacystic trophozoites). These organisms develop into the normal trophozoites when they become established in the large intestine.

Clinical Disease

The presentations of disease are seen with invasion of the intestinal mucosa and/or dissemination to other organs, the most common being the liver. However, it is estimated that only a small proportion (2 to 8%) of infected individuals will have invasive disease beyond the lumen of the bowel. Also, organisms may be spontaneously eliminated with no disease symptoms.

Asymptomatic Infection. Individuals harboring *E. histolytica* may have either a negative or weak antibody titer and negative stools for occult blood and may be passing cysts that can be detected if the routine ova and parasite examination is performed. Although trophozoites may also be found, they do not contain any phagocytized RBCs and cannot be differentiated from *E. dispar*. Isoenzyme analyses of organisms isolated from asymptomatic individuals generally indicate that the isolates belong to nonpathogens (*E. dispar*). Generally, asymptomatic patients never become symptomatic and may excrete cysts for a short period. This pattern is found for patients infected with either nonpathogenic or pathogenic species.

Intestinal Disease. Although the exact mode of mucosal penetration is not known, microscopy studies suggest that amebae have enzymes which lyse host tissue, possibly from lysosomes on the surface of the amebae or from ruptured organisms. Amebic ulcers most often develop in the cecum, appendix, or adjacent portion of the ascending colon; however, they can also be found in the sigmoidorectal area. From these primary sites, other lesions may occur. Ulcers are usually raised, with a small opening on the mucosal surface and a larger area of destruction below the surface, i.e., "flask shaped." The mucosal lining may appear normal between ulcers (Fig. 21.4).

The incubation period varies from a few days to a much longer time; however, in an area where *E. histolytica* is endemic it is impossible to determine exactly when the exposure took place. Normally, the time frame ranges from 1 to 4 weeks. Certainly not every patient infected with *E. histolytica* will develop invasive amebiasis; the outcome will depend on the interaction between parasite virulence factors and the host response.

There are four clinical forms of invasive intestinal amebiasis, all of which tend to be acute. They include dysentery or bloody diarrhea, fulminating colitis, amebic appendicitis, and ameboma of the colon. Dysentery and diarrhea account for 90% of cases of invasive intestinal amebiasis (11). Symptoms may range from none to those mimicking ulcerative colitis. Patients with colicky abdominal pain, frequent bowel movements, and tenesmus may present with a gradual onset of disease. With the onset of dysentery, bowel movements characterized by blood-tinged

TABLE 21.1 Intestinal protozoa: trophozoites of common amebae

Characteristic	***Entamoeba histolytica***	***Entamoeba dispar/ Entamoeba moshkovskii/ Entamoeba bangladeshi***	***Entamoeba hartmanni***	***Entamoeba coli***	***Endolimax nana***	***Iodamoeba bütschlii***
Size[a] (diam. or length)	12–60 µm (usual range, 15–20 µm); invasive forms may be over 20 µm	Same as *E. histolytica*	5–12 µm (usual range, 8–10 µm)	15–50 µm (usual range, 20–25 µm)	6–12 µm (usual range, 8–10 µm)	8–20 µm (usual range, 12–15 µm)
Motility	Progressive, with hyaline, fingerlike pseudopodia; motility may be rapid	Same as *E. histolytica*	Usually nonprogressive	Sluggish nondirectional, with blunt, granular pseudopodia	Sluggish, usually nonprogressive	Sluggish, usually nonprogressive
Nucleus: no. and visibility	Difficult to see in unstained preparations; 1 nucleus	Same as *E. histolytica*	Usually not seen in unstained preparations; 1 nucleus	Often visible in unstained preparations; 1 nucleus	Occasionally visible in unstained preparations; 1 nucleus	Usually not visible in unstained preparations; 1 nucleus
Peripheral chromatin (stained)	Fine granules, uniform in size and usually evenly distributed; may have beaded appearance	Same as *E. histolytica*	Nucleus may stain more darkly than in *E. histolytica*/*E. dispar*, although morphology is similar; chromatin may appear as solid ring rather than beaded	May be clumped and unevenly arranged on the membrane; may also appear as solid, dark ring with no beads or clumps	Usually no peripheral chromatin; nuclear chromatin may be quite variable	Usually no peripheral chromatin
Karyosome (stained)	Small, usually compact; centrally located but may also be eccentric	Same as *E. histolytica*	Usually small and compact; may be centrally located or eccentric	Large, not compact; may or may not be eccentric; may be diffuse and darkly stained	Large, irregularly shaped; may appear "blotlike"; many nuclear variations are common; may mimic *E. hartmanni* or *D. fragilis*	Large, may be surrounded by refractile granules that are difficult to see ("basket nucleus")
Cytoplasm appearance (stained)	Finely granular, "ground-glass" appearance; clear differentiation of ectoplasm and endoplasm; if present, vacuoles are usually small	Same as *E. histolytica*	Finely granular	Granular, with little differentiation into ectoplasm and endoplasm; usually vacuolated	Granular, vacuolated	Granular, may be heavily vacuolated
Inclusions (stained)	Noninvasive organism may contain bacteria; presence of RBCs diagnostic	Organisms usually contain bacteria; RBCs not present in cytoplasm	May contain bacteria; no RBCs	Bacteria, yeasts, other debris	Bacteria	Bacteria

[a] Wet preparation measurements (on permanent stains, organisms usually measure 1 to 2 µm less).

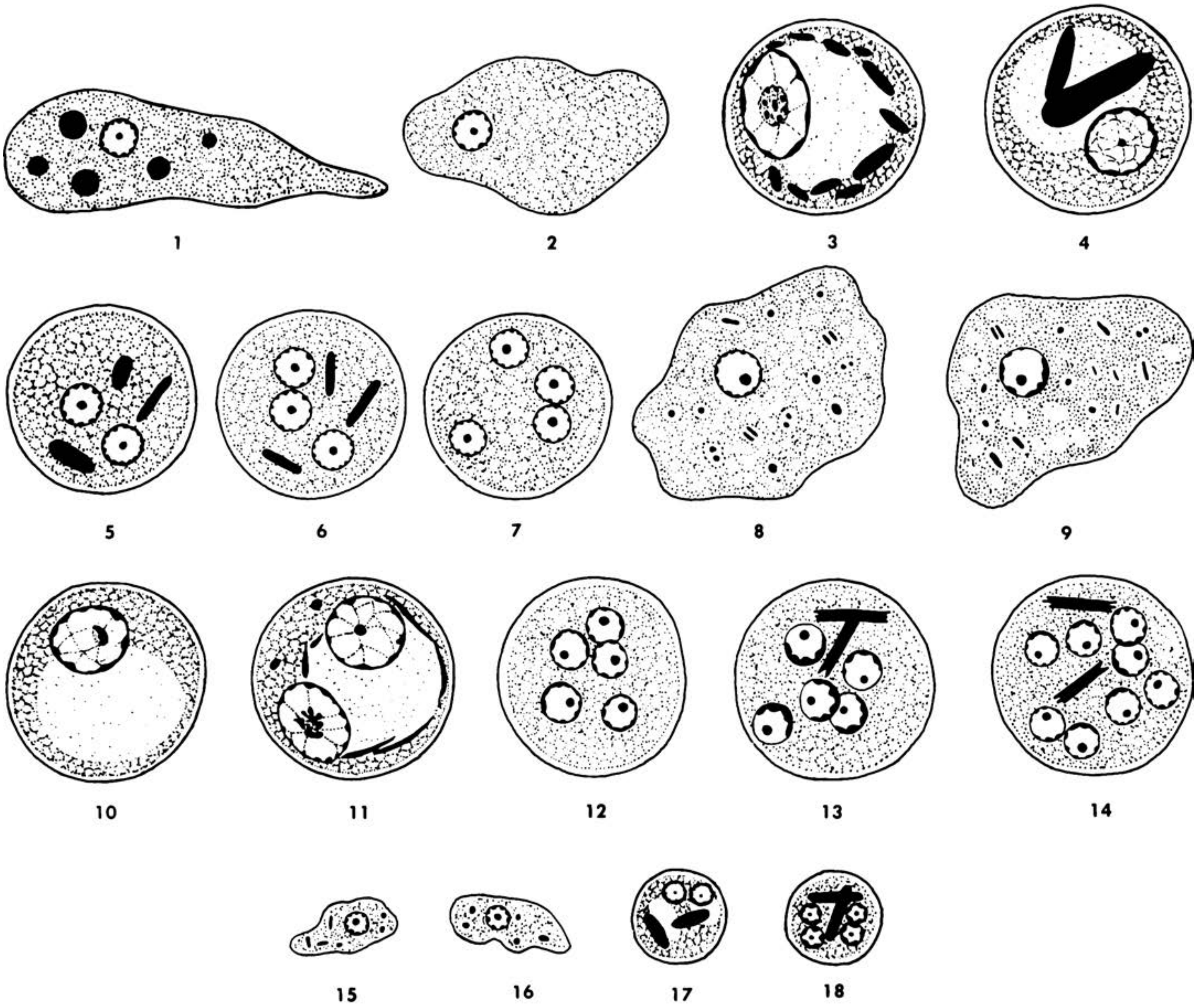
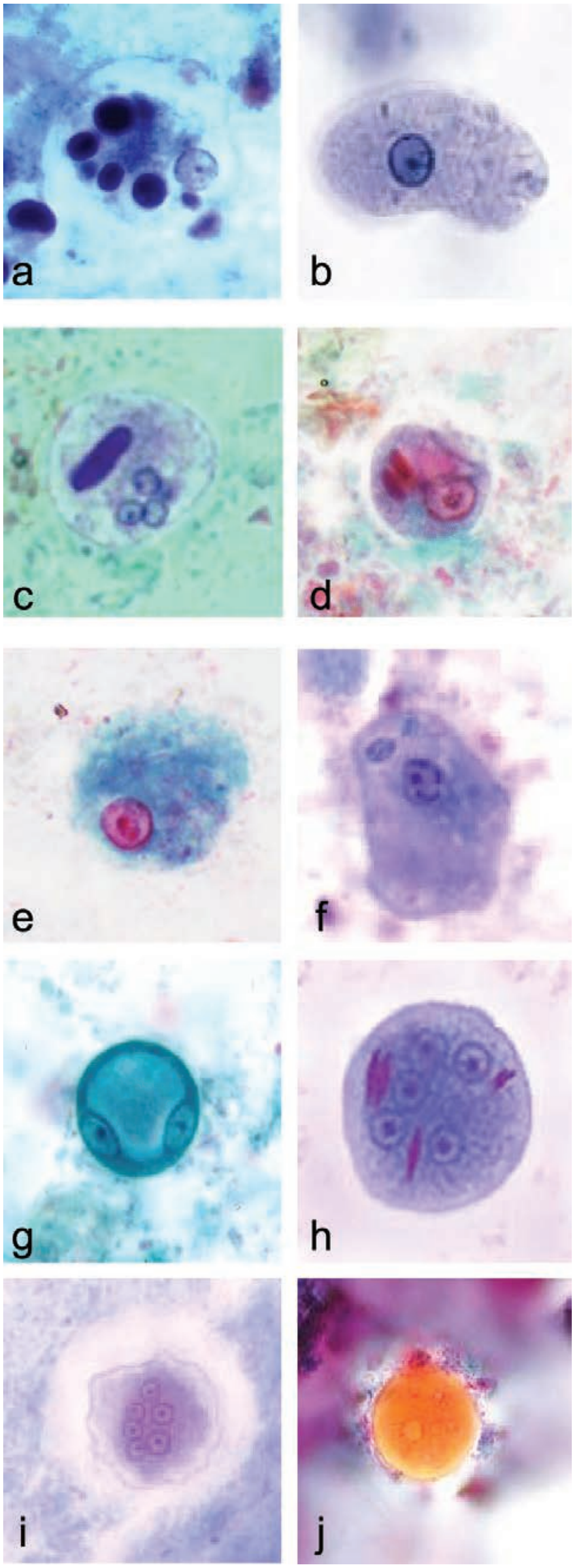


Figure 21.2 (1) Trophozoite of *Entamoeba histolytica*; (2) trophozoite of *E. histolytica*/*E. dispar*/*E. moshkovskii*; (3 and 4) early cysts of *E. histolytica*/*E. dispar*/*E. moshkovskii*; (5 to 7) cysts of *E. histolytica*/*E. dispar*/*E. moshkovskii*; (8 and 9) trophozoites of *Entamoeba coli*; (10 and 11) early cysts of *E. coli*; (12 to 14) cysts of *E. coli*; (15 and 16) trophozoites of *Entamoeba hartmanni*; (17 and 18) cysts of *E. hartmanni*. doi:10.1128/9781555819002.ch21.f2

mucus are frequent (up to 10 per day). Although dysentery may last for months, it usually varies from severe to mild over that time and may lead to weight loss and prostration. In patients with severe cases, symptoms may begin very suddenly and include profuse diarrhea (over 10 stools per day), fever, dehydration, and electrolyte imbalances. If the clinical picture is acute, the illness may mimic appendicitis, cholecystitis, intestinal obstruction, or diverticulitis.

Hepatic Disease. Blood flow draining the intestine tends to return to the liver, most commonly the upper right lobe (Fig. 21.5 and 21.6). The organisms present in the submucosa can be carried via the bloodstream to the liver. Onset of symptoms may be gradual or sudden; upper right abdominal pain with fever from 38 to 39°C is the most consistent finding. Weakness, weight loss, cough, and sweating are less commonly seen. There tends to be hepatomegaly with



tenderness; however, liver function tests may be normal or slightly abnormal, with jaundice being very rare. There may be changes at the base of the right lung owing to the elevated diaphragm. The abscess can be visualized radiologically, sonically, or by radionuclear scan, and the majority of patients have a single abscess in the right lobe of the liver. The most common complication is rupture of the abscess into the pleural space. An abscess can also extend into the peritoneum and through the skin. Hematogenous spread to the brain, as well as to the lungs, pericardium, and other sites, is possible. For patients living in areas of endemic infection and patients with a relevant travel history, amebic abscess should be suspected in those with spiking fever, weight loss, and abdominal pain in the upper right quadrant or epigastrium and those with tenderness in the liver area. The presence of leukocytosis, a high alkaline phosphatase level, and an elevated right diaphragm suggests a hepatic abscess. Pyogenic and amebic liver abscesses are the two most common hepatic abscesses. The severity of a pyogenic abscess depends on the bacterial source and the patient's underlying condition. Amebic abscess tends to be more prevalent in individuals with suppressed cell-mediated immunity, men, and younger individuals (13–15).

E. histolytica cysts and trophozoites are found in the stools of only a few patients with liver abscess. About 60% of these patients have no intestinal symptoms or any history of dysentery.

Although hepatic abscess is secondary to earlier colonization in the wall of the large intestine and is the most common site for extraintestinal amebiasis, *E. histolytica* has been seen in almost every soft organ and tissue of the body, including the lungs, heart, skin, and brain. Amebiasis of the lungs usually develops as an extension of a hepatic abscess that has ruptured through the diaphragm; however, it can also originate as a secondary site from the original infection in the intestine. Typically, the patient experiences expectoration of liver-colored sputum. In a very low percentage of cases, the pericardium can be involved; this is more likely to occur in patients with an abscess in the left lobe of the liver.

Figure 21.3 (a) Trophozoite of *Entamoeba histolytica*. (b) Trophozoite of *Entamoeba histolytica/E. dispar/E. moshkovskii*. (c and d) Cysts of *E. histolytica/E. dispar/E. moshkovskii/E. bangladeshi* (d is a precyst with a single nucleus). (e and f) Trophozoites of *Entamoeba coli*. (g to i) Cysts of *E. coli* (g is a precyst with two nuclei). Note the chromatoidal bars in panel h and the shrunken cyst (which is often seen in permanent stained smears) in panel i. Panel j represents an artifact that mimics an *E. coli* cyst (permanent stain). (Panel g is courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch21.f3

TABLE 21.2 Intestinal protozoa: cysts of common amebae

Characteristic	***Entamoeba histolytica/ Entamoeba dispar/ Entamoeba moshkovskii/ Entamoeba bangladeshi***	***Entamoeba hartmanni***	***Entamoeba coli***	***Endolimax nana***	***Iodamoeba bütschlii***
Size[a] (diam or length)	10–20 μm (usual range, 12–15 μm)	5–10 μm (usual range, 6–8 μm)	10–35 μm (usual range, 15–25 μm)	5–10 μm (usual range, 6–8 μm)	5–20 μm (usual range, 10–12 μm)
Shape	Usually spherical	Usually spherical	Usually spherical; may be oval, triangular, or other shapes; may be distorted on permanent stained slide because of inadequate fixative penetration	Usually oval, may be round	May vary from oval to round; cyst may collapse because of large glycogen vacuole space
Nucleus: no. and visibility	Mature cyst, 4; immature, 1 or 2 nuclei; nuclear characteristics difficult to see on wet preparation	Mature cyst, 4; immature, 1 or 2 nuclei; 2 nucleated cysts very common	Mature cyst, 8; occasionally 16 or more nuclei are seen; immature cysts with 2 or more nuclei are occasionally seen	Mature cyst, 4; immature cysts, 2; very rarely seen and may resemble cysts of *Enteromonas hominis*	Mature cyst, 1
Peripheral chromatin (stained)	Peripheral chromatin present; fine, uniform granules, evenly distributed; nuclear characteristics may not be as clearly visible as in trophozoite	Fine granules evenly distributed on the membrane; nuclear characteristics may be difficult to see	Coarsely granular; may be clumped and unevenly arranged on membrane; nuclear characteristics not as clearly defined as in trophozoite; may resemble *E. histolytica*/ *E. dispar*	No peripheral chromatin	No peripheral chromatin
Karyosome (stained)	Small, compact, usually centrally located but occasionally eccentric	Small, compact, usually centrally located	Large, may or may not be compact and/or eccentric; occasionally centrally located	Smaller than karyosome seen in trophozoites, but generally larger than those of the genus *Entamoeba*	Large, usually eccentric refractile granules may be on one side of karyosome ("basket nucleus")
Cytoplasm, chromatoidal bodies (stained)	May be present; bodies usually elongate, with blunt, rounded, smooth edges; may be round or oval	Usually present; bodies usually elongate, with blunt, rounded, smooth edges; may be round or oval	May be present (less frequently than in *E. histolytica*/*E. dispar*); splinter shaped with rough, pointed ends	Rare chromatoidal bodies present; occasionally small granules or inclusions seen; fine linear chromatoidals may be faintly visible on well-stained smears	No chromatoidal bodies present; small granules occasionally present
Glycogen (stained with iodine)	May be diffuse or absent in mature cyst; clumped chromatin mass may be present in early cysts (stains reddish brown with iodine)	May or may not be present as in *E. histolytica*/*E. dispar*	May be diffuse or absent in mature cyst; clumped mass occasionally seen in mature cysts (stains reddish brown with iodine)	Usually diffuse if present (stains reddish brown with iodine)	Large, compact, well-defined mass (stains reddish brown with iodine)

[a] Wet preparation measurements (on permanent stains, organisms usually measure 1 to 2 μm less).

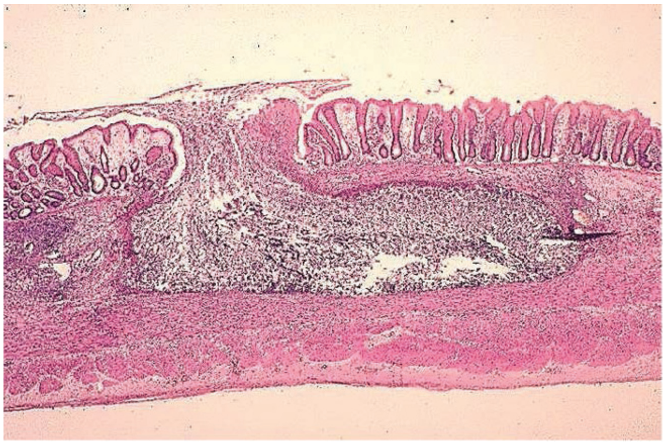
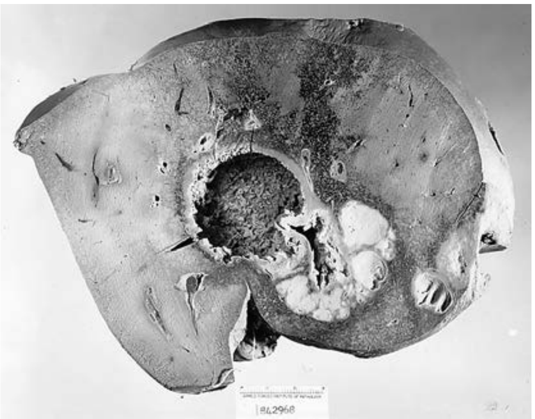


Figure 21.4 Amebic intestinal abscess. Note the "flask" shape (small opening on the mucosal surface and a larger area of destruction below the surface). (Courtesy of the CDC Public Health Image Library; Mae Melvin.)
doi:10.1128/9781555819002.ch21.f4

Cutaneous Disease. Cutaneous amebiasis (amebiasis cutis) is a rare disease, which occurs as a complication of amebic dysentery; however, it is a public health concern worldwide, particularly in developing nations. Amebiasis of the skin usually develops as a perianal extension of acute amebic colitis or on the abdominal wall due to rupture or open drainage of an internal intestinal or hepatic lesion (16). Cutaneous amebiasis may also occur as a venereal infection of the penis after anal intercourse (17). It is characterized by a severe clinical course, which can be confused with other disorders. Therefore, knowledge of its clinical features, histopathology, and pathogenesis is essential. Most patients are male (overall male to female ratio, 1.9 to 1). The disease presents as painful ulcers containing rare to many amebae, which can be detected microscopically using routine stains. It is recommended that tissue or smears be prepared and examined from the edges of the ulcer instead of the necrotic centers. Ingestion of RBCs by the trophozoites can be found and represents a diagnostic sign of the organism pathogenicity. Most cases resolve with the use of specific antiamebic drugs; however, if left untreated, progression is rapid and unrelenting, sometimes with massive destruction of skin and subcutaneous tissues. Therefore, cutaneous amebiasis is a particularly virulent form of amebiasis (18).

Figure 21.5 Liver containing multiple amebic abscesses. The necrotic tissue has been removed from one portion, leaving a cavity. Magnification, ×0.4. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch21.f5



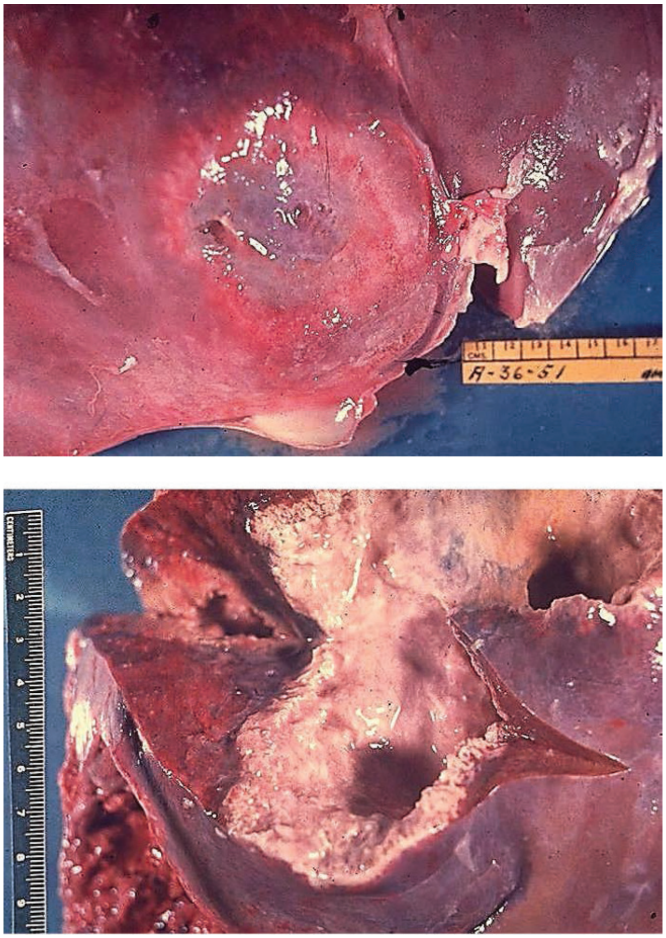


Figure 21.6 Amebic liver abscess. (Both images courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch21.f6

Brain abscess. Amebic abscess in the brain is quite rare and usually arises from either hepatic or pulmonary involvement (19). Brain abscess due to *E. histolytica* is most commonly seen in a small percentage of patients who also have an amebic liver abscess. After treatment for liver abscess with apparent improvement, the patient may develop symptoms and signs of central nervous system involvement. The average age of the patient is approximately

30, and symptoms include headache, vomiting, and altered mental status. Signs of amebic brain abscess include most commonly meningeal signs, facial nerve (VII) palsy, motor paralysis, and seizure. Although the cerebrospinal fluid may show some abnormalities, there is nothing that is disease-specific. Diagnosis is by serology and PCR of brain abscess aspirate or cerebrospinal fluid and treatment is with metronidazole. Certainly, brain involvement in amebic liver abscess should be suspected in patients with neurological signs and symptoms. Other rare ectopic sites include the spleen, adrenals, kidneys, ureters, urinary bladder, urethra, clitoris, and pericardium.

Pathogenesis

It has been estimated that *E. histolytica* infection kills more than 100,000 people each year. Key to the pathogenesis of this organism is its ability to directly lyse host cells and cause tissue destruction. Amebic lesions show evidence of cell lysis, tissue necrosis, and damage to the extracellular matrix. There is evidence that *E. histolytica* trophozoites interact with the host through a series of steps: adhesion to the target cell, phagocytosis, and cytopathic effect (11, 20). Numerous other parasite factors also play a role. From the perspective of the host, *E. histolytica* induces both humoral and cellular immune responses, with cell-mediated immunity representing the major human host defense against this complement-resistant cytolytic protozoan.

Our understanding of pathogenesis has expanded dramatically within the past few years. We now know that invasive strains of *E. histolytica* are resistant to complement-mediated lysis. These organisms activate complement via cleavage of C3 by an extracellular cysteine proteinase. Cysteine proteinases are important virulence factors encoded by at least three genes, one of which is unique to invasive strains (21). The amebic cysteine proteinases are homologous to proteinases released by transformed cells and probably represent a common mechanism of tissue invasion. The proteinase activity of amebae shows excellent correlation with their virulence. Cysteine proteinase can cleave the extracellular structural matrix and can degrade fibronectin and laminin, as well as type I collagen; this suggests that degradation of basement membrane is an important step in tissue invasion (21). Hemoglobin-degrading cysteine proteases also play an important role in iron acquisition by *E. histolytica*. Differences in the pathogenesis of *E. histolytica* and *E. dispar* also help explain the epidemiology, clinical syndromes, and pathology of amebiasis. Antibodies to the neutral cysteine proteinase have been detected in 83% of patients with invasive *E. histolytica* infections but not in patients with noninvasive *E. dispar* infections (22). Apparently, inhibition of the expression of cysteine proteinases does not affect *E. histolytica* cytopathic or hemolytic activity but inhibits phagocytosis. There is recent evidence that a cysteine protease (EhCP112) and a protein with an adherence domain (EhADH112) form the *E. histolytica* 112-kDa adhesin.

The first evidence of amebic pathology is depletion of the intestinal mucus and disruption of the epithelial barrier, which results from the action of cysteine proteinases as well as other factors (10, 21). Clinical isolates of *E. histolytica* in culture release 10- to 1,000-fold more cysteine proteinases than do *E. dispar* isolates. Trophozoites use cysteine proteinases to cleave and penetrate the extracellular matrix (collagen, elastin, fibrinogen, and laminin) in order to cause invasive disease (21). These enzymes also interfere with the host immune system; hence, the data supporting a key role of cysteine proteinases in virulence are quite compelling, especially since specific genes for cysteine proteinases appear to be present and overexpressed in *E. histolytica* and absent or underexpressed in *E. dispar*.

Several molecules that may be involved in the parasite-host interaction processes have been identified. A lectin inhibitable by galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc) is present on the trophozoite surface. Adherence of *E. histolytica* trophozoites to colonic mucin, epithelium, and other target cells is mediated by the amebic Gal/GalNAc lectin (24). Lectin activity in the parasite is regulated by inside-out signaling; this inside-out signaling via the lectin cytoplasmic domain may control the extracellular adhesive activity of the amebic lectin and provides evidence of the role of lectin in virulence (25). Studies also confirm that dominant-negative mutations in the Gal/GalNAc lectin affect adhesion and cytolysis while mutations in meromyosin affect cytoskeletal function (26). Myosin IB also is involved in phagocytosis of human erythrocytes by *E. histolytica* (27). Recently, a distinct β_2 integrin-like molecule with a potential role in cellular adherence was identified. This amebic β_2 integrin appeared to be distinct from the amebic Gal/GalNAc lectin based on recombinant expression, amebic colocalization, and enzyme-linked immunosorbent assay (ELISA) studies. The presence of integrin-dependent binding may allow trophozoites to opportunistically adhere to activated intestinal epithelium or vascular endothelium during amebic colitis or hepatic abscess (28).

There are also correlations between differences in the cell surface phosphorylated glycolipids and virulence; lipophosphoglycan and lipophosphopeptidoglycan molecules are abundant in virulent strains of *E. histolytica* and are absent or present at low levels in avirulent *E. histolytica* strains and *E. dispar* (29).

Various amebic pore-forming proteins are known, of which the 5-kDa protein (amebapore) has been extensively studied. These proteins can insert into the lipid bilayers of target cells, forming ion channels (11).

Data obtained by using human myeloid cells as target cells *in vitro* have revealed that the target cells die due to necrosis with cell swelling, rupture of plasma membranes, and release of cell contents including nucleic acids. These findings confirm the notion that these particular target cells undergo necrosis when killed by viable trophozoites, as well as by isolated amebapores (11).

The role of zinc in the pathogenicity of *E. histolytica* may also be relevant. Apparently, zinc alters the functionality of the amebae *in vitro* by causing a decrease in replication and adhesion and *in vivo* by inhibiting amebic pathogenicity.

Diagnosis

Routine Diagnostic Procedures. The laboratory diagnosis of amebiasis, particularly intestinal amebiasis, depends on a number of procedures, any one of which can prevent organism recovery if not performed properly (Table 21.3). Organism detection depends on collection of the correct specimens, the number of specimens submitted, processing methods and diagnostic tests used, and examination by personnel who are well trained in identification of protozoa. This diagnosis can be one of the more difficult to achieve. Lack of appropriate training and diagnostic testing may lead to missed infections. In some cases, false positives may be due to identification of human white blood cells as amebae. A complete discussion of specimen collection and submission to the laboratory is presented in chapter 2.

The standard ova and parasite examination is the recommended procedure for recovery and identification of *E. histolytica* in stool specimens. Microscopic examination of a direct saline wet mount may reveal motile trophozoites, which may contain RBCs. However, the number of times these trophozoites with RBCs are present is limited. In many patients who do not present with acute dysentery, trophozoites may be present but do not contain RBCs and the organisms may be *E. histolytica* or *E. dispar*. An asymptomatic individual may have few trophozoites and possibly only cysts in the stool. Although the concentration technique is helpful in demonstrating cysts, *the most important technique for the recovery and identification of protozoan organisms is the permanent stained smear* (normally stained with trichrome or iron hematoxylin). A minimum of three specimens collected over a time frame of not more than 10 days is recommended (30–32).

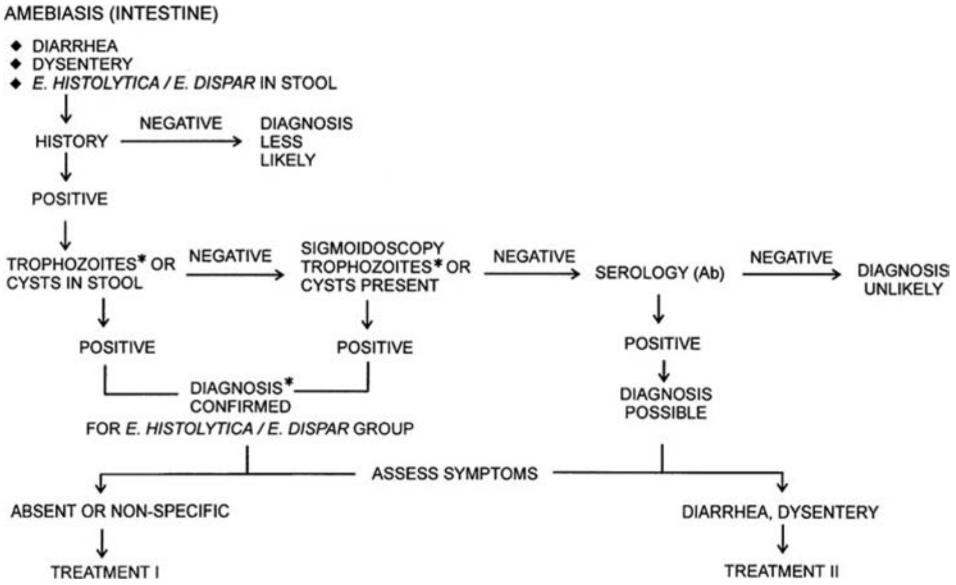
Note that if the permanent stained smear is quite dark, a delicate *E. histolytica*/*E. dispar* organism may appear more like *Entamoeba coli*; on a very thin, pale smear, *E. coli* can appear more like *E. histolytica*/*E. dispar*. Also, when the organisms are dying or have been poorly fixed, they appear more highly vacuolated, thus looking like *E. coli* trophozoites rather than *E. histolytica*/*E. dispar*.

If slides are prepared properly and examined carefully, sigmoidoscopy specimens may be very helpful. At least six areas of the mucosa should be sampled. Smears from these areas should be examined after permanent staining. This does not take the place of the recommended minimum of three stool specimens submitted for ova and parasite examinations (direct, concentration, and permanent stained smear). Refer to Algorithm 21.1 for detailed information.

TABLE 21.3 Intestinal amebae: recommended diagnostic procedures

Organism	Recommended specimen	Diagnostic procedure[a]
Entamoeba histolytica	Stool	Complete ova and parasite exam
		Immunoassays for antigen detection
	Sigmoidoscopy material	Permanent stain
	Hepatic abscess aspirate	Digestion/wet preparations
Entamoeba dispar	Stool	Complete ova and parasite exam
Entamoeba moshkovskii	Stool	Complete ova and parasite exam
Entamoeba bangladeshi	Stool	Complete ova and parasite exam
Entamoeba hartmanni	Stool	Complete ova and parasite exam
Entamoeba coli	Stool	Complete ova and parasite exam
Entamoeba polecki	Stool	Complete ova and parasite exam
Entamoeba gingivalis	Gingival scrapings	Wet preparations and permanent stains
	Pyorrheal material	Wet preparations and permanent stains
Endolimax nana	Stool	Complete ova and parasite exam
Iodamoeba bütschlii	Stool	Complete ova and parasite exam
Blastocystis spp.	Stool	Complete ova and parasite exam

[a] Differentiation among *E. histolytica*, *E. dispar*, *E. moshkovskii*, and *E. bangladeshi* requires molecular means; morphology is basically the same for all (unless *E. histolytica* trophozoites containing ingested RBCs are seen).


AMEBIASIS (INTESTINE)
◆ DIARRHEA
◆ DYSENTERY
◆ *E. HISTOLYTICA / E. DISPAR* IN STOOL
HISTORY
NEGATIVE
DIAGNOSIS LESS LIKELY
POSITIVE
TROPHOZOITES* OR CYSTS IN STOOL
NEGATIVE
SIGMOIDOSCOPY TROPHOZOITES* OR CYSTS PRESENT
NEGATIVE
SEROLOGY (Ab)
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
POSITIVE
POSITIVE
DIAGNOSIS* CONFIRMED FOR *E. HISTOLYTICA / E. DISPAR* GROUP
DIAGNOSIS POSSIBLE
ASSESS SYMPTOMS
ABSENT OR NON-SPECIFIC
TREATMENT I
DIARRHEA, DYSENTERY
TREATMENT II

Algorithm 21.1 Intestinal amebiasis. Fecal immunoassay kits can be used on human stool specimens to identify organisms in the group *E. histolytica*/*E. dispar* or to differentiate *E. histolytica* from *E. dispar*. Currently available reagents require fresh or frozen specimens only. *, trophozoites containing RBCs confirmatory for *E. histolytica*; trophozoites with no ingested RBCs and/or cysts confirm only the *E. histolytica*/*E. dispar* group.

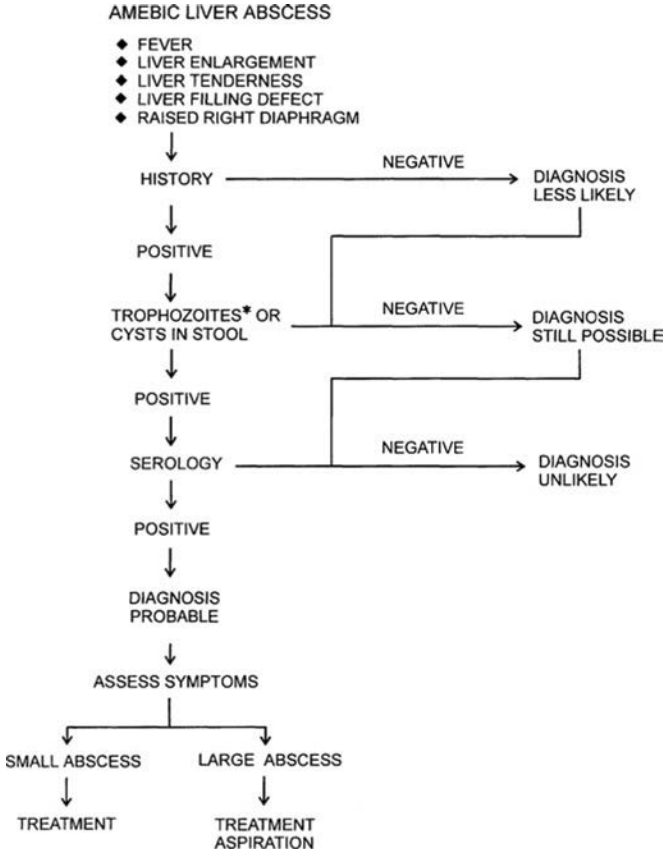
doi:10.1128/9781555819002.ch21.Alg21.1

Definitive diagnosis of liver abscess can be achieved by identification of organisms from liver aspirate material. However, this procedure is rarely performed, and often the specimen obtained is not collected properly. Aspirated material must be aliquoted into several different containers as it is removed from the abscess; amebae may be found only in the last portion of the aspirated material, theoretically material from the abscess wall, not necrotic debris from the abscess center. Refer to Algorithm 21.2 for detailed information.

Antigen Detection. A number of enzyme immunoassay reagents are commercially available, and their specificity and sensitivity provide excellent options for the clinical laboratory (Table 21.4). These tests can determine the presence of the *E. histolytica*/*E. dispar* group as being distinct from the rest of the *Entamoeba* species such as nonpathogenic *E. coli* or *E. hartmanni*. Other test reagents can actually separate *E. histolytica* from *E. dispar*. The majority of these procedures use the ELISA or enzyme immunoassay (EIA) formats (33). However, in some cases they have been found to be less sensitive than PCR and in other instances they have been found equal to PCR. Another product is available as a cartridge format that uses an immunochromatographic strip-based detection system for the *E. histolytica*/*E. dispar* group, *G. lamblia*, and *Cryptosporidium* spp. (3). These commercially available reagent kits require fresh or frozen stools; formalin has been found to interfere with the *Entamoeba* reagents. Any available antigen detection system should always be reviewed for compatibility with stools submitted in preservatives rather than fresh specimens. There are limitations when using kits that include organisms in the genus *Entamoeba* (3). However, almost all reagent kits for the detection of *G. lamblia* and *Cryptosporidium* spp. can be used with formalin-based stool preservatives, the Universal Fixative (TOTAL-FIX), as well as fresh or frozen specimens.

To provide clinically relevant information to physicians for treatment of patients infected with pathogenic E. *histolytica*, methods involving monoclonal antibodies, purified antigens, or DNA probes are now available. Reagents have been developed to differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar* (33). Although some of these reagents are now available, routine use in clinical laboratories may not be accepted because of cost, limited numbers of cases, and infrequent identification of organisms in the genus *Entamoeba*. Reliable probes based on the hypothesis of zymodeme stability and two separate species (one pathogenic and one nonpathogenic) offer significant improvements over current serologic or direct detection methods; these probes may also help eliminate false-positive results due to misidentification of human cells or other nonpathogenic protozoa in patient specimens.

The laboratory now has the ability to routinely indicate to the physician whether the *E. histolytica*/*E. dispar* organisms seen in the stool specimen are pathogenic *E. histolytica* or nonpathogenic *E. dispar*. Without the use of these types of reagents, the only way to morphologically identify true pathogenic *E. histolytica* would be to observe the rare presence of trophozoites containing ingested RBCs. In the event the laboratory does not use these reagents, the presence of *E. histolytica*/*E. dispar* should be reported to the physician, along with some additional commentary



Algorithm 21.2 Amebic liver abscess. *, trophozoites containing RBCs confirmatory for *E. histolytica*; trophozoites with no ingested RBCs and/or cysts confirm only the *E. histolytica*/*E. dispar* group. doi:10.1128/9781555819002.ch21.Alg21.2

related to the newer information on pathogenicity. Ultimately, the physician will have to decide on the issue of therapy on the basis of the patient's clinical condition. However, without more accurate information on the pathogenicity of organisms seen in clinical specimens, from a public health point of view it may be appropriate to treat all infected patients. Also, depending on each state's requirements, pathogenic *E. histolytica*, as well as *E. histolytica*/*E. dispar*, is generally reported to the public health facility (county).

Antibody Detection. Serologic testing for intestinal disease is normally not recommended unless the patient has true dysentery; even in these cases, the titer (indirect hemagglutination as an example) may be low and thus difficult to interpret. The definitive diagnosis of intestinal amebiasis should not be made without demonstrating the organisms. For patients suspected of having extraintestinal disease, serologic tests are much more relevant (33). Indirect hemagglutination and indirect fluorescent-antibody tests have been reported positive with titers of ≥1:256 and ≥1:200, respectively, in almost 100% of cases of amebic liver abscess (3). Positive serologic results, in addition to clinical findings, make the diagnosis highly probable. In the absence of STAT serologic tests for amebiasis (tests with very short turnaround times for results), the decision about the identity of the causative agent often must be made on clinical grounds and on the basis of results of other diagnostic tests such as scans.

Serologic tests based on recombinant *E. histolytica* antigens, using SREHP and the 170-kDa subunit of the galactose-specific adhesin, have been developed. More than 50% of the patients examined had become seronegative by one or both recombinant tests within 180 days of their diagnosis of amebic liver abscess. In contrast, all patients remained seropositive by a standard conventional indirect-hemagglutination test at more than 6 months after presentation (34). These findings may make the recombinant-antigen tests more useful for the serologic diagnosis of amebiasis in areas of endemic infection.

In a study in Bangladesh, 199 (69%) of children tested were positive at least once using the *E. histolytica* antigen detection test. Although the incidence rate was not related to baseline nutritional status, age, sex, blood group, or duration of breast feeding, it was significantly related to the baseline *E. histolytica* anti-lectin IgG status of the children. Children who had recovered from a diarrheal episode with *E. histolytica*, but not *E. dispar*, had half the chance of developing subsequent *E. histolytica*-associated diarrhea, consistent with the development of species-specific acquired immunity (35).

In another study in Brazil, 30% of a sample population was colonized with *E. histolytica*. However, no correlation between seropositivity for anti-GalNAc lectin antibody and colonization was found. These results suggest that colonization does not necessarily produce immunity to reinfection (36).

Histology. A histologic diagnosis of amebiasis can be made when the trophozoites within the tissue are identified. Organisms must be differentiated from host cells, particularly histiocytes and ganglion cells. Periodic acid-Schiff staining is often used to help locate the organisms. The organisms appear bright pink with a green-blue background (depending on the counterstain used). Hematoxylin and eosin staining also allows the typical morphology to be seen, thus allowing accurate identification. As a result of sectioning, some organisms exhibit the evenly arranged nuclear chromatin with the central karyosome and some no longer contain the nucleus.

TABLE 21.4 Commercial assays (antigen, antibody, molecular): *E. histolytica*

Assay	Manufacturer	Sensitivity (%)	Specificity (%)	Comments[a]
Antigen				
Antigen detection TechLab *E. histolytica* II	TechLab/Alere, Blacksburg, VA	96.9–100	~95	EIA; requires fresh or frozen stool; reagents differentiate between *E. histolytica* and *E. dispar*
Antigen detection TechLab *E. histolytica* Quik Chek	TechLab/Alere, Blacksburg, VA	98	100	Rapid; requires fresh or frozen stool; reagents differentiate between *E. histolytica* and *E. dispar*
ProSpecT *Entamoeba histolytica* microplate assay	Remel/Thermo Scientific, Lenexa, KS	90.3	97.7	EIA; identification of the *E. histolytica*/*E. dispar* group; fresh or preserved specimens acceptable
Entamoeba CELISA-*PATH*	Cellabs Pty Ltd., Brookvale, Australia	95–100	93–100	EIA; no specific information per fresh or preserved specimens
Wampole *E. histolytica* test	Wampole Laboratories, Cranbury, NJ	94.7		EIA; requires fresh or frozen stool; reagents differentiate between *E. histolytica* and *E. dispar*
Merlin Optimun S ELISA	Merlin Diagnostika, Bernheim-Hersel, Germany	81.8	99.2	EIA; no specific information per fresh or preserved specimens
RIDASCREEN Entamoeba	R-Biopharm AG, Darmstadt, Germany	81.8	99.2	EIA
Triage parasite panel	BIOSITE Diagnostics, San Diego, CA	~83–96	~99–100	Lateral-flow cartridge; identification of the *E. histolytica*/*E. dispar* group; fresh or frozen specimens required
Antibody				
Entamoeba IHA	Biotrin Int., Heidelberg, Germany			EIA
Antibody detection IHA Cellognost Amoebiasis	Behring Diagnostics, Marburg, Germany	~72–100	91–99	IHA
Light Diagnostics Amebiasis (*Entamoeba histolytica*) EIA	Chemicon, Int., Temecula, CA	95	97	EIA; USA
Amebiasis Serology Microwell EIA	IVD Research, Carlsbad, CA	92.5–97.9	91.3–94.8	EIA; USA
BLA-Bichrolatex-Amibe	Fumouze Diagnostics, Levallois-Perret, France	93.3–98.3	95.5–96.1	LA
Ambiase HAI FUMOUZE	Fumouze Diagnostics, Levallois-Perret, France	93.4	97.5	IHA
RIDASCREEN *Entamoeba* IgG	R-Biopharm AG, Darmstadt, Germany	97.7-100	95.6-97.4	EIA
Molecular				
RIDAGENE Stool Panel PCR (*Giardia*, *Cryptosporidium*, *E. histolytica*, *Dientamoeba*)	R-Biopharm AG, Darmstadt, Germany	(<5 DNA copies per reaction)		PCR
xTAG Gastrointestinal Pathogen Panel (xTAG GPP), viral, bacterial, parasitic pathogens (*Giardia*, *Cryptosporidium*, *E. histolytica*)	Luminex, Austin, TX	87.5–100	95–100	Multiplexed nucleic acid test
BD MAX Enteric Parasite Panel (*Giardia*, *Cryptosporidium*, *E. histolytica*)	Becton, Dickinson and Co., Sparks, MD	Limited data to date	Limited data to date	PCR

(continued)

TABLE 21.4 *(continued)*

Assay	Manufacturer	Sensitivity (%)	Specificity (%)	Comments[a]
BioFire FilmArray Gastrointestinal Panel, bacterial, viral, parasitic pathogens (*Cryptosporidium*, *Cyclospora*, *E. histolytica*, *Giardia*)	bioMérieux, Marcy l'Etoile, France	Limited data to date	Limited data to date	PCR

[a] EIA, enzyme immunoassay; IHA, indirect hemagglutination; LA, latex agglutination; PCR, polymerase chain reaction. (Adapted from references 1, 3, 19, 34, 47, and 68.)

KEY POINTS—LABORATORY DIAGNOSIS

Entamoeba histolytica

1. When routine ova and parasite examinations are ordered, three specimens (stool) should be submitted for the diagnosis of intestinal amebiasis; fecal fixatives are highly recommended to eliminate the lag time between specimen passage and receipt/preservation in the laboratory.
2. Any examination for parasites in stool specimens must include the use of a permanent stained smear (even on formed stool).
3. Presumptive identification on a wet preparation must be confirmed by using the permanent stained smear; wet mounts are recommended on liquid or very runny specimens. If the specimen is submitted in fixative, no direct mount is required (no organism motility would be visible with the use of a fixative). Without using *E. histolytica*-specific antigen detection tests, the presence of the organism(s) should be reported as follows. Trophozoites containing ingested RBCs within the cytoplasm should be reported as the true pathogen ("*Entamoeba histolytica* trophozoites present"). However, if cysts or trophozoites containing no ingested RBCs are seen, the report should indicate that the *Entamoeba* group is present ("*Entamoeba histolytica/E. dispar* group trophozoites and cysts present").
4. The six smears for permanent staining should be prepared at sigmoidoscopy but should not take the place of the ova and parasite examination.
5. Immunoassay procedures can be performed to confirm the presence of the *E. histolytica/E. dispar* group or can be used to differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*. Remember that some of these reagent kits may require fresh or frozen stools only; formalin-preserved stool and stool containing polyvinyl alcohol are not acceptable.
6. The serologic test result for antibody may or may not be positive in intestinal disease and is much more likely to be positive in extraintestinal disease (liver abscess).

Treatment

Over the years, there has been much controversy concerning the possibility of pathogenic and nonpathogenic strains of *E. histolytica* and whether every patient found to harbor this parasite should be treated. Brumpt is credited with the first suggestion that *E. histolytica* consists of two morphologically identical organisms, one pathogenic and invasive (*E. histolytica*) and the other a harmless commensal (*E. dispar*). While carriers usually harbor nonpathogenic *E. dispar*, pathogenic *E. histolytica* also may be found in these individuals. At present, tests which routinely differentiate between *E. histolytica* and *E. dispar* are not used routinely by all diagnostic laboratories. The diagnosis of *E. histolytica/E. dispar* infection is most often based on organism morphology. For this reason, in general, patients in the United States who harbor these organisms may be treated, regardless of the presence or absence of symptoms.

There are two classes of drugs used in the treatment of amebic infections: luminal amebicides such as iodoquinol or diloxanide furoate, and tissue amebicides such as metronidazole, chloroquine, or dehydroemetine. Because differences in drug efficacy exist, it is important that the laboratory report for the physician indicate whether cysts, trophozoites, or both are present in the stool specimen (37).

Asymptomatic Infection. Patients found to have true *E. histolytica* in the intestinal tract, even if they are asymptomatic, should be treated to eliminate the organisms. Both diloxanide furoate and iodoquinol or paromomycin are available for treatment of patients who have cysts in the lumen of the gut. A study encompassing 14 years of experience of using diloxanide furoate for treating asymptomatic cyst passers in the United States indicates that the drug is safe and effective and may be particularly well tolerated in children (38). In general, these treatments are ineffective against extraintestinal disease. If the patient is passing both trophozoites and cysts, the recommended treatment is metronidazole plus iodoquinol (38).

Mild to Moderate Disease. For patients with mild to moderate disease, metronidazole (Flagyl) should be used when tissue invasion occurs, regardless of the tissue involved. Drugs directed against the lumen organisms should also be used in these instances.

Severe Intestinal Disease. Metronidazole plus one of the luminal drugs should be used for therapy.

Hepatic Disease. Metronidazole plus one of the luminal drugs should be used to treat hepatic disease. Some other combinations can also be used; some contain emetine, in which case the patient must be monitored very carefully for possible cardiotoxicity. The importance of using both luminal and tissue amebicides to treat patients with amebic liver abscess is emphasized. Asymptomatic colonization may be present with the true pathogen, *E. histolytica*. In patients treated with metronidazole (tissue amebicide), there is generally 100% clinical response to the hepatic lesions; however, failure to eliminate the organism from the bowel can lead to second bouts with invasive disease and intestinal colonization. Also, these carriers constitute a public health hazard since they are shedding infective cysts. Specific drugs and dosages are discussed in chapter 36.

Epidemiology and Prevention

Infections with *E. histolytica* are worldwide in distribution and are generally most prevalent in the tropics. In 1984, 500 million people were estimated to be infected with *E. histolytica*, and 40 to 50 million of these had extensive symptoms including colitis or extraintestinal abscesses. It is estimated that there are 100,000 deaths each year. Prevalence figures for the United States are generally thought to be <5%. It has also been estimated that for every case of invasive disease diagnosed, there are at least 10 to 20 asymptomatic individuals excreting infective cysts.

Population groups with a higher incidence of amebiasis include recent immigrants and refugees from South and Central America and from Southeast Asia. Residents in southeastern and southwestern parts of the United States also tend to have more infections with intestinal parasites, as do groups such as patients in mental institutions.

Following broad social changes seen in the late 1960s, the open expression of homosexuality, increased numbers of sexual contacts, increased frequency of sexual activities, and anonymity of sexual partners contributed to dramatic increases in the prevalence of sexually transmitted organisms, including *E. histolytica*. Although infections with this organism are usually associated with poor sanitation and underdeveloped areas of the world, epidemiologic studies in both Europe and the United States in the 1970s documented that sexual transmission of amebiasis occurred mainly among urban homosexual men. With the increase in sexually transmitted pathogens, a number of clinical syndromes were recognized; these clinical presentations were grouped together and referred to as "the gay bowel syndrome." Many published reports confirm that *E. histolytica* is one of the major pathogens in the gay bowel syndrome; however, this term is no longer commonly used. It is also well known that the clinical presentations within the homosexual community often differ from those seen in the heterosexual population.

Epidemiologic evidence of sexual transmission of *E. histolytica* has grown significantly since the early 1970s, particularly in areas such as New York City and San Francisco. In San Francisco, the incidence of reported symptomatic intestinal amebiasis among homosexual men between 20 and 39 years of age has increased over 1,000% during the last 10 years. Although percentages vary, it appears that approximately 30% of urban homosexual men may be infected with *E. histolytica*, a sharp increase over the estimated rate of less than 5% seen in the general population within the United States.

Direct oral-anal contact (anilingus) is a common practice among homosexual men, leading to both fecal exposure and oral contact with a variety of intestinal pathogens. Although anilingus has often been listed as a key risk factor in potential exposure, transmission can also occur during oral-genital sex after anal intercourse has occurred. It has also been shown that active heterosexuals can acquire infection with *E. histolytica* through sexual activities that provide an opportunity for fecal-oral contamination. The key factor is not necessarily homosexuality but the frequency of sexual activity and the potential for fecal-oral contact.

It is important to remember than in earlier studies in which "*E. histolytica*" was specifically mentioned, reference is actually being made to the *E. histolytica*/*E. dispar* group rather than the actual pathogen, *E. histolytica*. Genotyping of *E. histolytica* isolates could serve as a tool to fingerprint individual isolates, thus helping to determine geographic origins of isolates and routes of transmission. Molecular tools are now available not only to diagnose *Entamoeba* species accurately but also to study intraspecies genetic diversity (39).

With the advent of AIDS and the subsequent modifications in sexual practices within homosexual communities, the incidence of *E. histolytica* infection is thought to be much lower than before. In recent years, the coccidian parasites *Cystoisospora* (*Isospora*) *belli* and *Cryptosporidium* spp. and the microsporidia *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* have become much more of a problem in patients with AIDS.

There are certain urban areas (Mexico City, Mexico; Medellin, Colombia; and Durban, South Africa) where the incidence of invasive disease is considerably higher than in the rest of the world. Contributing factors (not well delineated) may include poor nutrition, tropical climate, decreased immunologic competence of the host, stress, altered bacterial flora in the colon, traumatic injuries to the colonic mucosa, alcoholism, and genetic factors.

Humans are the reservoir host for *E. histolytica* and can transmit the infection to other humans, primates, dogs, cats, and possibly pigs. However, contact with sewage-contaminated water provides another route of infection; therefore, amebiasis can be considered a zoonotic waterborne infection as well as being transmitted through human-to-human transmission (40). The cyst stages are very resistant to environmental conditions and can remain viable in the soil for 8 days at 28 to 34°C, 40 days at 2 to 6°C, and 60 days at 0°C. Cysts are normally removed by sand filtration or destroyed by 200 ppm of iodine, 5 to 10% acetic acid, or boiling. The asymptomatic cyst passer who is a food handler is generally thought to play the most important role in transmission.

A colonization-blocking vaccine could eliminate *E. histolytica* as a cause of human disease, particularly since humans serve as the only significant reservoir host (41). The application of molecular biological techniques has led to the identification and structural characterization of three amebic antigens, SREHP, the 170-kDa subunit of the Gal/GalNAc binding lectin, and the 29-kDa cysteine-rich protein, which all show promise as recombinant antigen-based vaccines to prevent amebiasis (41). An immunogenic dodecapeptide derived from the SREHP molecule has been genetically fused to the B subunit of cholera toxin to create a recombinant protein capable of inducing both antiamebic and anti-cholera toxin antibodies when administered by the oral route. Continued research should lead to the development of a cost-effective oral combination "enteric-pathogen" vaccine capable of inducing protective mucosal immune responses to several important enteric diseases including amebiasis (42).

Entamoeba dispar

In the past, an unexplained aspect of the epidemiology of amebiasis was the number of asymptomatic individuals who were passing cysts but had no clinical evidence of invasive disease. Extensive work involving isoenzyme analysis and molecular techniques has demonstrated conclusively that there are two genetically distinct but morphologically identical species of what was formerly known as *E. histolytica*. After many years of debate, the species that is associated with amebiasis in humans is now classified as *E. histolytica*. The other species, which is more common but is not capable of causing invasive disease, is called *E. dispar*. Studies confirming the differences between the two organisms include direct sequencing of the PCR-amplified small-subunit rRNA gene of *E. dispar* and the design of primers for rapid differentiation from *E. histolytica*; detection of differences in the phosphoglucomutases and cysteine proteinases from *E. histolytica* and *E. dispar*; molecular biology of the differences in the hexokinase isoenzyme pattern; detection of differences in the secretion of acid phosphatase; and detection of DNA sequences unique to *E. histolytica* (6, 7, 11, 43, 45, 46).

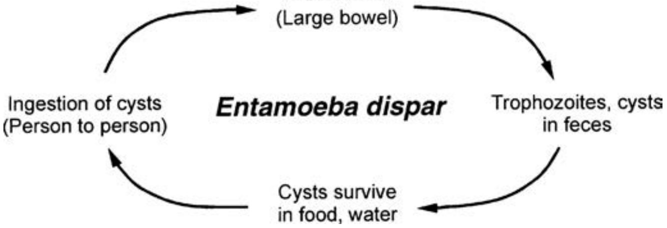
Life Cycle and Morphology

The life cycle is essentially identical to that of *Entamoeba coli* or any of the other nonpathogenic intestinal protozoa, and the cyst form is the infective form for humans (Fig. 21.7).

Morphology of Trophozoites (Table 21.1). Living trophozoites vary in diameter from about 12 to 60 μm. Motility has been described as rapid and unidirectional, with pseudopods forming quickly in response to the conditions around the organism; it may appear to be sporadic. Although this characteristic is often described, it is rare to diagnose these organisms on the basis of motility seen in a direct wet mount. The cytoplasm is differentiated into a clear outer ectoplasm and a more granular inner endoplasm. Based on the recent ability to culture these organisms in axenic culture systems and on light and electron microscopy studies, there may be some morphologic differences between *E. histolytica* and *E. dispar*. However, these differences would not be recognized by routine diagnostic methods such as the permanent stained smear. If organisms were seen that were consistent with *E. histolytica*/*E. dispar* on the permanent stained smear, the laboratory report would indicate that fact and would be written as "*Entamoeba histolytica*/*E. dispar*" or "*Entamoeba histolytica*/*E. dispar* group or complex."

When the organism is examined on a permanent stained smear (trichrome or iron hematoxylin), the morphological characteristics are easily seen. The nucleus generally has evenly arranged chromatin on the nuclear membrane and has a small, compact, centrally located karyosome. The cytoplasm is described as finely granular with few ingested bacteria or debris in vacuoles. Ingested RBCs are never seen in the trophozoites; if ingested RBCs are seen, this finding

Figure 21.7 Life cycle of *Entamoeba dispar*, the same as *E. moshkovskii* and *E. bangladeshi*.
doi:10.1128/9781555819002.ch21.f7


HUMANS
(Large bowel)
Trophozoites, cysts in feces
Cysts survive in food, water
Ingestion of cysts (Person to person)
Entamoeba dispar

identifies the organism as the true pathogen *E. histolytica*, not *E. dispar*.

Morphology of Cysts (Table 21.2). Within the immature cyst, there may be two types of inclusions, a glycogen mass and highly refractile chromatoidal bars with smooth, rounded edges. As the cysts mature, nuclear division occurs with the production of four nuclei; the cysts range in diameter from 10 to 20 μm. Usually, as the cyst matures, the glycogen disappears and chromatoidal bars may or may not be present. Cyst morphology does not differentiate between *E. histolytica* and *E. dispar*. Cyst formation occurs only within the intestinal tract; once the stool has been passed from the body, cyst formation does not occur. The one-, two-, and four-nucleated cysts are infective and represent the mode of transmission from one host to another (Fig. 21.1 to 21.3).

Clinical Disease

Prior to the separation of *E. histolytica* into two distinct species (*E. histolytica* and *E. dispar*), it was suggested that some *E. histolytica* strains were pathogenic and some were not. Now that *E. dispar* has been classified as a nonpathogenic organism, totally separated from the pathogenic *E. histolytica*, treatment is usually not recommended. However, this recommendation is based on the separation of the two species by molecular means, not by morphology alone. Since few laboratories will be routinely identifying the two *Entamoeba* species to the level of *E. histolytica* or *E. dispar*, the clinician will have to decide on the basis of clinical findings whether the patient has true pathogenic *E. histolytica* or nonpathogenic *E. dispar* infection. The laboratory report will merely indicate the presence of the "*Entamoeba histolytica*/*E. dispar*" group.

Diagnosis

Routine Diagnostic Procedures. Infection with *E. dispar* cannot be diagnosed on the basis of morphologic criteria alone, even from the permanent stained smear. When the organism is seen in the routine ova and parasite examination, it cannot be differentiated from *E. histolytica* unless trophozoites are seen to contain RBCs in the cytoplasm, a finding that is diagnostic for *E. histolytica*. Using routine diagnostic methods and not immunoassay procedures specific for *E. histolytica*, morphologic criteria would provide characteristics of both; the laboratory report would indicate "*Entamoeba histolytica*/*E. dispar*."

Antigen Detection. Immunoassay procedures are available that can determine the presence of the *E. histolytica*/*E. dispar* group as being distinct from the rest of the *Entamoeba* species, such as nonpathogenic *E. coli* or *E. hartmanni*. Other test reagents can actually separate *E. histolytica* from *E. dispar*. Most of these procedures use the ELISA or EIA formats (46). In some cases, they have been found to be less sensitive than PCR (15), and in other instances they have been found equal to PCR (15, 43). Another product that is available as a cartridge format uses an immunochromatographic strip-based detection system for the *E. histolytica*/*E. dispar* group, *G. lamblia*, and *Cryptosporidium* spp. (3). These commercially available reagent kits require fresh or frozen stools; formalin has been found to interfere with the *Entamoeba* reagents. Any available antigen detection system should always be reviewed for compatibility with stools submitted in preservatives rather than fresh specimens.

Epidemiology and Prevention

The mode of transmission is similar to that found with other protozoa and is related to the ingestion of infective cysts in contaminated food or water. Based on differences in numbers between symptomatic patients with amebiasis (*E. histolytica*) and those who remain asymptomatic (*E. dispar*), it is obvious that many more patients are infected with nonpathogenic *E. dispar* than with pathogenic *E. histolytica*. Since travel from temperate zones to tropical and subtropical areas continues to become more common, it is necessary to consider imported amebiasis in many areas of the world where the infection is normally seen less frequently than infections with the nonpathogenic *E. dispar*.

Entamoeba moshkovskii

Entamoeba moshkovskii is found worldwide and is generally considered to be a free-living ameba. Based on microscopic morphology, this organism is indistinguishable from *E. histolytica* and *E. dispar*, except in cases of invasive disease when *E. histolytica* contains ingested RBCs. Although first isolated from sewage, *E. moshkovskii* can also be found in clean riverine sediments to brackish coastal pools. Apparently, there are some differences that separate this organism from *E. histolytica* and *E. dispar*. However, these differences pertain to physiology rather than morphology: *E. moshkovskii* is osmotolerant, can be cultured at room temperature, and is resistant to emetine (40).

Life Cycle and Morphology

The life cycle is essentially identical to that of *E. dispar*, and morphological differences are minimal to none (Fig. 21.7). In wet preparations, trophozoites usually range in size from 15 to 20 μm and cysts normally range in size from 12 to 15 μm. It is important to remember that on the permanent stained smear there is a certain amount of artificial shrinkage due to dehydration; therefore, all of the organisms, including pathogenic *E. histolytica*, may be somewhat smaller (from 1 to 1.5 µm) than the sizes quoted for the wet-preparation measurements.

Morphology of Trophozoites (Table 21.1). Trophozoites do not ingest RBCs, and the motility is similar to that of both *E. histolytica* and *E. dispar* (Fig. 21.3). Nuclear and cytoplasmic characteristics are very similar to those seen in *E. histolytica*; however, trophozoites of *E. moshkovskii* do not contain ingested RBCs.

Morphology of Cysts (Table 21.2). Nuclear characteristics and chromatoidal bars are similar to those in *E. histolytica* and *E. dispar* (Fig. 21.3).

Clinical Disease

Human isolates of *E. bangladeshi* have been recovered from asymptomatic patients, as well as those with diarrhea. It remains unclear what impact these organisms may have on the normal gut flora. Studies similar to those undertaken with *E. moshkovskii* may help to clarify the overall pathogenicity of *E. bangladeshi* and relevant symptoms attributed to this organism. Since many organisms within the genus *Entamoeba* exhibit almost identical morphology, patient clinical isolates may need to be identified using molecular methods.

Diagnosis

Because *E. moshkovskii* can be easily confused with other amebae, particularly in a wet preparation, it is almost mandatory that the final identification be obtained from the permanent stained smear. However, without the use of molecular tools to specifically identify this organism, the results are usually reported as "*Entamoeba histolytica*/*E. dispar* group" with no mention of *E. moshkovskii*. The true prevalence of this organism may be much higher than suspected; this may also explain some of the microscopy-positive/antigen-negative results obtained when using the *Entamoeba* test kit.

Epidemiology and Prevention

The mode of transmission is similar to that for other protozoa and is related to ingestion of cysts in contaminated food or water. In areas where accurate identifications have been made, the prevalence of *E. moshkovskii* may be similar to or higher than that of *E. histolytica* (40). Using PCR techniques, in one study from Bangladesh *E. dispar*-infected children were almost twice as likely to have a mixed infection with *E. moshkovskii* (35%) than with *E. dispar* and *E. histolytica* (18%) or with *E. dispar* alone (18%) (39). Future epidemiologic studies of *E. histolytica* infection should include the capability of diagnosing all three species individually (*E. histolytica*, *E. dispar*, and *E. moshkovskii*) (48).

Entamoeba bangladeshi

Microscopy and culture for the genus *Entamoeba* have limited specificity because several species, which vary in their pathogenic potential, have morphologically similar cysts and trophozoites (49). In 2010–2011, during analysis of feces positive for *Entamoeba* organisms by microscopy or culture but negative for *E. histolytica*, *E. dispar*, and *E. moshkovskii* by PCR, a new species was identified, which was named *Entamoeba bangladeshi* nov. sp. in recognition of the support of the Bangladesh community for this research (4). The sequenced clones from two different isolates, one diarrheal and one surveillance specimen, were completely novel when compared with the small subunit rRNA gene sequences from other organisms and did not match any previously sequenced *Entamoeba* species. These isolates represent a new species of *Entamoeba* (GenBank accession nos. JQ412861 and JQ412862), here named *E. bangladeshi*.

Life Cycle and Morphology

The life cycle is essentially identical to that of *E. dispar* and *E. moshkovskii* and morphological differences are minimal to none (Fig. 21.7). In wet preparations, trophozoites usually range in size from 15 to 20 µm and cysts normally range in size from 12 to 15 µm. It is important to remember that on the permanent stained smear there is a certain amount of artificial shrinkage due to dehydration; therefore, all of the organisms, including pathogenic *E. histolytica*, may be somewhat smaller (from 1 to 1.5 µm) than the sizes quoted for the wet-preparation measurements. Overall, by light microscopy, no apparent differences between *E. bangladeshi* and *E. histolytica* were detected. The physical resemblance between *E. histolytica* and *E. bangladeshi* is a diagnostic problem because direct microscopic examination of fecal samples is still used as a diagnostic tool to detect *E. histolytica* in areas in which these species are endemic.

Morphology of Trophozoites (Table 21.1). Trophozoites do not ingest RBCs, and the motility is similar to that of *E. histolytica*, *E. dispar*, and *E. moshkovskii* (Fig. 21.3). Nuclear and cytoplasmic characteristics are very similar to those seen in *E. histolytica*; however, trophozoites of *E. bangladeshi* do not contain ingested RBCs.

Morphology of Cysts (Table 21.2). Nuclear characteristics and chromatoidal bars are similar to those in *E. histolytica*, *E. dispar*, and *E. moshkovskii* (Fig. 21.3).

Clinical Disease

Human isolates of *E. bangladeshi* have been recovered from asymptomatic patients, as well as those with diarrhea.

It remains unclear what impact these organisms may have on the normal gut flora. Studies similar to those undertaken with *E. moshkovskii* may help to clarify the overall pathogenicity of *E. bangladeshi* and relevant symptoms attributed to this organism. Since many organisms within the genus *Entamoeba* exhibit almost identical morphology, patient clinical isolates may need to be identified using molecular methods.

Diagnosis

E. bangladeshi is microscopically indistinguishable from *E. histolytica* in cyst and trophozoite stages (4). In xenic culture, *E. bangladeshi* has the ability to grow at 37°C and room temperature, a characteristic shared with *E. moshkovskii* and *E. ecuadoriensis* (environmental isolate) but which distinguishes it from *E. histolytica* and *E. dispar*. Tests were negative in *E. histolytica* ELISA and in species-specific PCRs. Currently, *E. bangladeshi* is only identifiable by its small subunit rRNA gene sequence. The positive specimens were obtained from infant feces in Mirpur, Dhaka, Bangladesh, N 23°4734″, E 90°2138″ (4).

Epidemiology and Prevention

The mode of transmission is similar to that for other protozoa and is related to ingestion of cysts in contaminated food or water. *E. bangladeshi* is grouped with the clade of *Entamoeba* infecting humans, including *E. histolytica*. *E. bangladeshi*, however, appears to be more distantly related than the noninvasive *E. dispar*, but closer than *E. moshkovskii*, to *E. histolytica*. Future epidemiologic studies of *E. histolytica* infection should include the capability of diagnosing all four species individually (*E. histolytica*, *E. dispar*, *E. moshkovskii*, and *E. bangladeshi*).

Entamoeba hartmanni

Entamoeba hartmanni is found worldwide and until 1957 was generally considered to be a small race of *E. histolytica*. Consequently, the prevalence figures prior to that time are thought to be inaccurate. It is now well accepted that this ameba is a separate species from *E. histolytica*.

Life Cycle and Morphology

The life cycle is essentially identical to that of *E. dispar*, and morphological differences involve size, although there is even an overlap in size between the two species (Fig. 21.8). In wet preparations, trophozoites range in size from 4 to 12 µm and cysts range in size from 5 to 10 µm. It is important to remember that on the permanent stained smear there is a certain amount of artificial shrinkage due to dehydration; thus, all of the organisms, including pathogenic *E. histolytica*, may be somewhat smaller (from 1 to 1.5 µm) than the sizes quoted for the wet-preparation measurements.

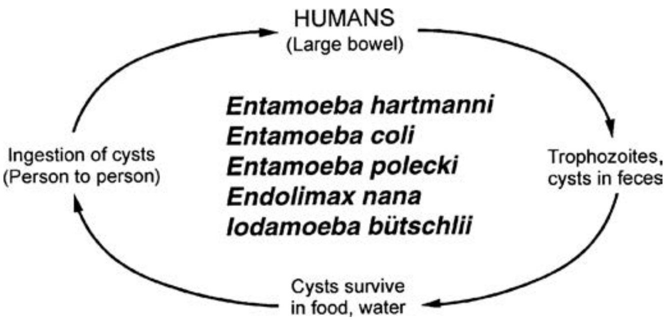
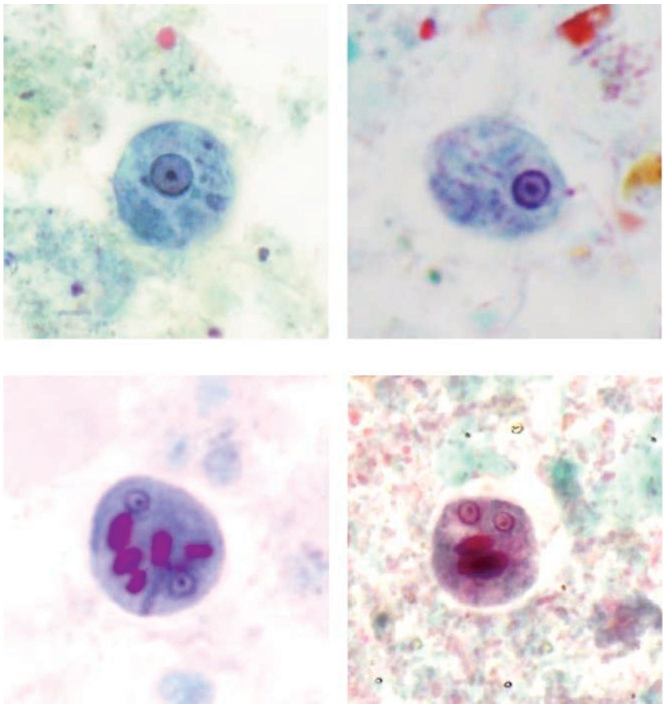


Figure 21.8 Life cycle of *Entamoeba hartmanni*, *Entamoeba coli*, *Entamoeba polecki*, *Endolimax nana*, and *Iodamoeba bütschlii*. doi:10.1128/9781555819002.ch21.f8

Morphology of Trophozoites (Table 21.1). Trophozoites do not ingest RBCs, and the motility is usually less rapid (Fig. 21.2 and 21.9). The trophozoites measure 5 to 12 µm (usual range, 8 to 10 µm). The nuclear and cytoplasmic characteristics are very similar to those seen in *E. histolytica*, with two exceptions. Frequently, cysts contain only one or two nuclei, even though the mature cyst contains four nuclei. Mature cysts of *E. hartmanni* also tend to retain

Figure 21.9 (Upper) Trophozoites of *Entamoeba hartmanni*; (lower) cysts of *E. hartmanni*. (Note that cysts often contain only two nuclei, instead of the mature four nuclei.) doi:10.1128/9781555819002.ch21.f9



their chromatoidal bars, a characteristic which is often not seen in *E. histolytica*/*E. dispar*.

Morphology of Cysts (Table 21.2). Chromatoidal bars are similar to those in *E. histolytica* and *E. dispar* but are smaller and more numerous (Fig. 21.2 and 21.9). Because differentiation between *E. hartmanni* and *E. histolytica*/*E. dispar* at the species level depends primarily on size, it is mandatory that laboratories use calibrated microscopes which are rechecked periodically for accuracy. The cysts measure 5–10 μm (usual range, 6–8 μm).

Clinical Disease

Although early studies incriminated *E. hartmanni* as a possible animal pathogen and suggested that it could transform from small "nonpathogenic" strains to large "pathogenic" ones, none of this information has been adequately confirmed by subsequent studies. Because *E. hartmanni* is accepted as being nonpathogenic, treatment is usually not recommended. However, presence of the organism is always reported; nonpathogens are acquired via fecal/oral contamination by the patient, the same way the pathogens are acquired. Thus, the report indicates the patient has ingested something contaminated with fecal material.

Diagnosis

Because *E. hartmanni* can be easily confused with other small amebae, particularly in a wet preparation, it is almost mandatory that the final identification be obtained from the permanent stained smear. Accurate measurement of organisms also confirms the tentative visual diagnosis (Table 21.3).

Epidemiology and Prevention

The mode of transmission is similar to that found with other protozoa and is related to ingestion of cysts in contaminated food or water. In areas where accurate identifications have been made, the prevalence of *E. hartmanni* is similar to that of *E. histolytica*.

Entamoeba coli

Although *Entamoeba coli* was probably recognized and described in the late 1800s, it was not until 1913 that the organism was proven to be nonpathogenic in human volunteers. *E. coli* is worldwide in distribution. Like *E. histolytica*, it tends to be more common in the warmer climates, but it has also been found in colder areas such as Alaska.

Life Cycle and Morphology

The life cycle is identical to that of *E. dispar* (Fig. 21.7).

Morphology of Trophozoites (Table 21.1). Living trophozoites tend to be somewhat larger than those of *E. histolytica* or *E. dispar* and range from 15 to 50 μm (Fig. 21.2 and 21.3). Motility has been described as sluggish with broad, short pseudopods. It may be extremely difficult to differentiate nonpathogenic *E. coli* from pathogenic *E. histolytica* in wet preparations. On the permanent stained smear, the cytoplasm appears granular with few to numerous vacuoles containing bacteria, yeasts, and other food materials. The nucleus has a moderately large karyosome that is frequently eccentric in position. The chromatin on the nuclear membrane is usually clumped and irregular in placement. If there are RBCs in the intestinal tract, *E. coli* may ingest these rather than bacteria; occasionally, the cytoplasm also contains ingested spores of *Sphaerita* sp. and possibly a *G. lamblia* cyst.

Morphology of Cysts (Table 21.2). Again, for reasons that are not specifically known, trophozoites discharge their undigested food and begin to round up prior to precyst and cyst formation. Early cysts usually contain a dense glycogen mass and may also contain chromatoidal bars, which tend to be splinter shaped and irregular. Eventually, the nuclei divide until the mature cyst, containing eight nuclei, is formed (Fig. 21.2 and 21.3). Occasionally, the number of nuclei reaches 16. The cysts measure 10 to 35 μm and almost always lose their chromatoidal bars as they mature. For some reason, as the cyst of *E. coli* matures, it becomes more resistant to fixation with various preservatives. Therefore, the cyst may be seen on the wet preparation but not on the permanent stained smear. Occasionally, on trichrome smears, the cysts appear distorted, shrunk, and somewhat pink. This is not an indication of poor reagents or techniques but, rather, an indication that these cysts do not fix well with the routinely used preservatives. Better fixation and more detailed morphology can be obtained by heating some of the fixatives prior to specimen preservation, although for most laboratories, this is not a practical approach.

Studies suggest that after cyst ingestion, the metacyst undergoes division of the cytoplasm, thus becoming metacystic trophozoites that will grow and divide within the lumen of the intestine. It is interesting that the total number of trophozoites formed from the mature cyst usually is fewer than eight.

Diagnosis

With the exception of the mature cyst, the morphologies of *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. bangladeshi*, and *E. coli* are similar in most of the stages. Therefore, it is very important to examine permanent stained smears, even if a tentative identification has been made from a wet preparation examination. Specific treatment is not recommended for this nonpathogen (Table 21.3). Consequently, correct differentiation between the five species is critical to good patient care. Since the five species are acquired by the patient in the same way, it is also important to remember that all can be found in the same patient. If few *E. histolytica*/*E. dispar* organisms are present among many *E. coli* organisms, additional searching and/or the use of species-specific immunoassay testing may be necessary to correctly identify the different species.

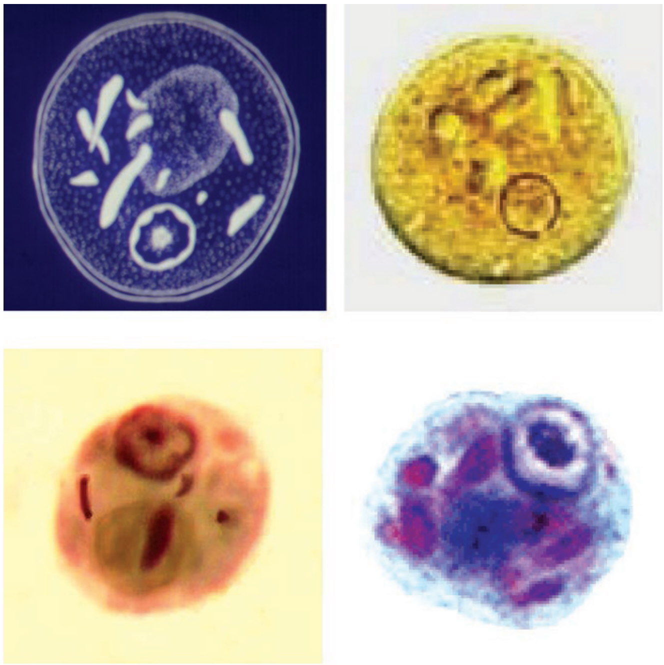
Epidemiology and Prevention

The mode of transmission is ingestion of cysts from contaminated food or water. Apparently, the infection is readily acquired, and in some warmer climates or areas with primitive hygiene conditions, the infection rate can be quite high. Prevention depends on adequate disposal of human excreta and improved personal hygiene (preventive measures which apply to the majority of the intestinal protozoa).

Entamoeba polecki

Entamoeba polecki was originally found in the intestines of pigs and monkeys and has also been found as a human parasite (Fig. 21.10). In certain areas of the world, such as Papua New Guinea, it is the most common intestinal ameba of humans. Few cases are reported, possibly in part because the organism resembles *E. histolytica*/*E. dispar*, *E. moshkovskii*, *E. bangladeshi*, and *E. coli*. The trophozoites resemble *E. coli* in that the cytoplasm is granular, containing ingested bacteria, and the motility tends to be sluggish (Table 21.5). The nuclear morphology is almost a composite of those of *E. histolytica*/*E. dispar*, *E. moshkovskii*, *E. bangladeshi*, and *E. coli*. Without some of the cyst stages for comparison, it would be very difficult to identify this organism to the species level on the basis of the trophozoite alone. The cyst normally has only a single nucleus, chromatoidal material like that seen in *E. histolytica*, and frequently an inclusion body (Fig. 21.10; Table 21.6). This mass tends to be round or oval and is not sharply defined on the edges. The material, which is not glycogen, remains on the permanent stained smear and stains less intensely than nuclear material or chromatoidal bars. Differentiation of this organism from either *E. histolytica* or *E. coli* is rarely accomplished on the basis of a wet preparation examination. The size on the permanent stained smear would range from 10 to 12 µm for the trophozoite and from 5 to 11 µm for the cyst. To date, there is no evidence of pathogenicity in humans; therefore, no therapy is recommended.

Figure 21.10 (Upper, left) Cyst of *Entamoeba polecki* (illustration) (note the large inclusion in the cytoplasm as seen in the drawing); (right) cyst of *E. polecki* (direct wet mount with iodine; large vacuole is more difficult to see; however, chromatoidal bars are present). (Lower, left and right) *E. polecki* cyst forms (the large inclusion is visible in both organisms).
doi:10.1128/9781555819002.ch21.f10



Entamoeba gingivalis

Entamoeba gingivalis was the first parasitic ameba of humans to be described. It was recovered from the soft tartar between the teeth. It has also been recovered from the tonsillar crypts and can multiply in bronchial mucus, thus appearing in sputum (Fig. 21.11). Since morphologically it is very similar to *E. histolytica*, it is important to make the correct identification from a sputum specimen—that is, *E. gingivalis*, which is considered to be a nonpathogen—rather than *E. histolytica* from a possible pulmonary abscess. The trophozoite measures approximately 5 to 15 µm, and the cytoplasm most often contains ingested leukocytes (Table 21.5). On the permanent stained smear, nuclear fragments of the white cells can be seen within the food vacuoles, which are usually larger than the vacuoles seen in *E. histolytica* (Fig. 21.11). This fact will help to differentiate the two, since *E. gingivalis* is the only species that ingests white cells. No cysts are formed by this species (Table 21.6). Although these amebae are most often recovered from patients with pyorrhea alveolaris, *E. gingivalis* is still considered to be nonpathogenic (49).

Organisms identified as *E. gingivalis* have been recovered in vaginal and cervical smears from women using intrauterine devices (IUDs); the organisms spontaneously disappeared after removal of the devices. However, more recent information has implicated this organism as causing symptoms in several clinical situations. A prospective study conducted in Egypt determined that *E. gingivalis* was

TABLE 21.5 Intestinal protozoa: trophozoites of less common amebae

Characteristic	*Entamoeba polecki*	*Entamoeba gingivalis*
Size (diam or length)	10–12 μm	5–20 μm (avg, 10–15 μm)
Motility	Usually nonprogressive, sluggish (like *E. coli*)	Multiple pseudopodia, vary from long and lobose to short and blunt
Nucleus: no. and visibility	Can occasionally be seen on a wet preparation; intermediate between *E. histolytica* and *E. coli*; 1 nucleus	Similar to *E. histolytica*, 1 nucleus
Peripheral chromatin (stained)	Fine granules (may be interspersed with large granules) evenly arranged on membrane; chromatin may also be clumped at one or both edges of membrane	Fine granules, closely packed
Karyosome (stained)	Small, usually centrally located	Small, well defined, usually centrally located
Cytoplasm appearance (stained)	Finely granular	Finely granular
Inclusions (stained)	May contain ingested bacteria	Ingested epithelial cells and host leukocytes

found in 12.64% of IUD users and in 6.9% of nonusers ($P > 0.3$). It was found that 90.9% of those harboring *E. gingivalis* in their genital tract had the parasite in their oral cavity. In the infected cases, vaginal discharge was more common (81.8%) than in noninfected cases (32.9%), and such difference was statistically significant ($P < 0.05$). Also, the authors found excessive vaginal discharge was more common than backache and menorrhagia in the infected cases. Higher incidence of *E. gingivalis* infection in IUD users is related to oral cavity infection, residence, the facility where the inserted IUD was inserted, and hand washing or nonwashing before checking the strings (50).

In an unusual case, *E. gingivalis* was identified in a left upper neck nodule by fine-needle aspiration. Apparently, the patient had an increased number of amebae within the oral cavity secondary to radiation therapy. Radiation therapy may have contributed to a fistula tract between the oral cavity and the surgical incision site, thus resulting in the formation of a small inflammatory nodule in the upper portion of the neck (51).

Another unusual case has been reported, *E. gingivalis* in acute osteomyelitis of the mandible, following bisphosphonate therapy for multiple myeloma. However, identification of the organism was not confirmed using molecular methods, but morphology only (52).

E. gingivalis has also been reported in pulmonary computed tomography-guided fine-needle aspiration material, from a 60-year-old man with a lung mass. The mechanism of pulmonary infection in this case is not known. However, a pulmonary polymicrobial environment probably contrib-

TABLE 21.6 Intestinal protozoa: cysts of less common amebae

Characteristic	*Entamoeba polecki*	*Entamoeba gingivalis*
Size (diam or length)	5–11 μm	No known cyst stage
Shape	Usually spherical	
Nucleus: no. and visibility	Mature cyst, 1; may be visible in wet preparations (rarely 2 or 4 nuclei)	
Peripheral chromatin (stained)	Similar to that seen in trophozoite	
Karyosome	Similar to that seen in trophozoite	
Cytoplasm (chromatoidal bodies, glycogen, inclusions [stained])	Abundant, angular pointed ends; threadlike chromatoidals may be present; half of cysts contain spherical or ovoid “inclusion mass”	

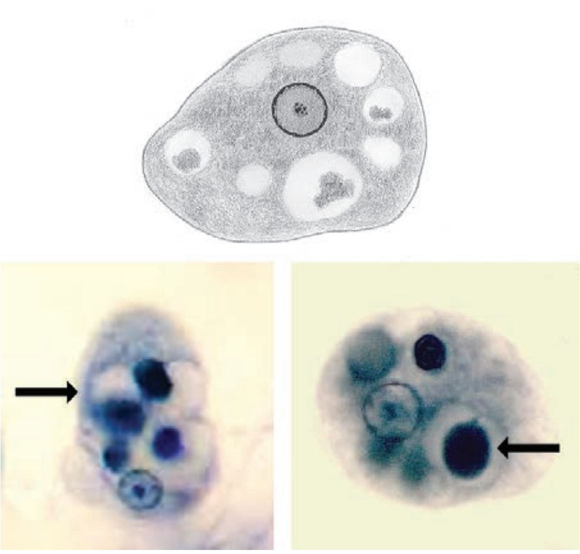


Figure 21.11 (Upper) Trophozoite of *Entamoeba gingivalis* (illustration by Sharon Belkin). (Lower, left and right) Trophozoites of *E. gingivalis* (note the ingested polymorphonuclear leukocytes—arrows). Both stained with iron-hematoxylin.
doi:10.1128/9781555819002.ch21.f11

uted to this clinical condition, secondary to aspiration pneumonia (53).

Although *E. gingivalis* is considered a nonpathogen, it has been found in the oral cavities of HIV-1-infected patients with periodontal disease. Its presence was not related to the degree of immunodeficiency but to the HIV diagnosis (54).

Endolimax nana

Endolimax nana is one of the smaller nonpathogenic amebae and was distinguished as a separate ameba around 1908. It is also worldwide in distribution and is seen in most populations at least as frequently as *E. coli*.

Life Cycle and Morphology

E. nana has the same stages in the life cycle as does *E. dispar*: trophozoite, precyst, cyst, and metacystic forms (Fig. 21.8). The trophozoite usually measures from 6 to 12 µm, with the normal range being 8 to 10 µm (Table 21.1).

Motility has been described as sluggish and nonprogressive with blunt, hyaline pseudopods. In the permanent stained smear, the nucleus is more easily seen. There is normally no peripheral chromatin on the nuclear membrane, and the karyosome tends to be large, with either a central or eccentric location within the nucleus (Fig. 21.12 and 21.13). There is tremendous nuclear variation in this organism, and occasionally it mimics *Dientamoeba fragilis* and *E. hartmanni*. The more organisms there are on the smear, the more likely it is that some of them will mimic other species of the amebae. The cytoplasm may have small vacuoles containing ingested debris or bacteria.

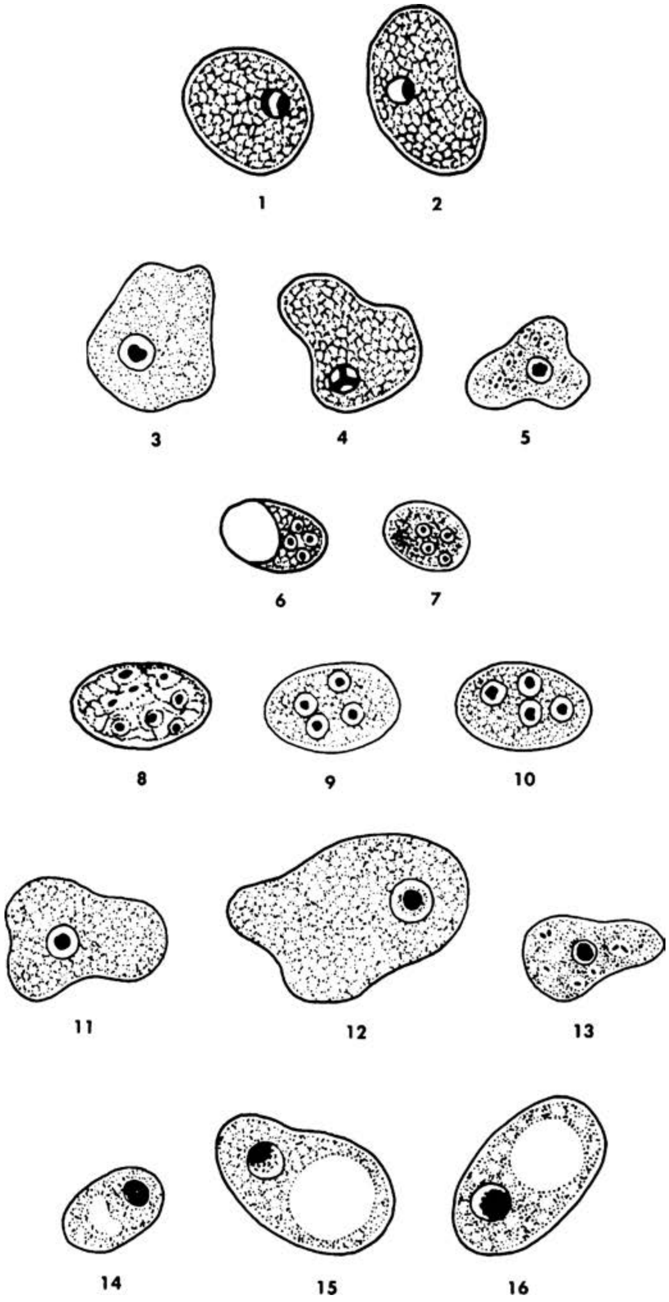


Figure 21.12 (1 to 5) Trophozoites of *Endolimax nana*; (6 to 10) cysts of *E. nana*; (11 to 13) trophozoites of *Iodamoeba bütschlii*; (14 to 16) cysts of *I. bütschlii*.
doi:10.1128/9781555819002.ch21.f12

Cysts usually measure 5 to 10 µm, with a normal range of 6 to 8 µm (Table 21.2). In some instances, cysts as large as 14 µm have been seen. The cyst is usually oval to round, with the mature cyst containing four nuclei. Occasionally, very small, slightly curved chromatoidal bars are present

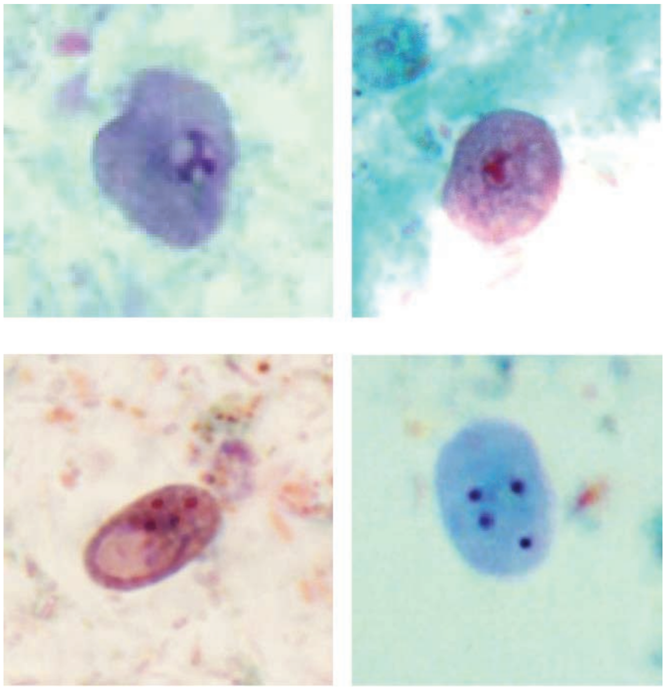


Figure 21.13 (Upper, left and right) Trophozoites of *Endolimax nana* (note the nuclear variation in both organisms). (Lower) Cysts of *E. nana*, containing four karyosomes with no peripheral chromatin; (left) note the vacuole. This can be confused with an *I. bütschlii* cyst; however, an *E. nana* cyst contains four nuclei, not just one. doi:10.1128/9781555819002.ch21.f13

(Fig. 21.12). It is unusual to see the two-nucleated stage, and there are frequently both trophozoites and cysts in clinical specimens. In some cases, there may be a large vacuole within the cyst (Fig. 21.13); although the vacuole might be suggestive of *Iodamoeba bütschlii*, *E. nana* cysts contain four nuclei, not one.

Diagnosis

Although *E. nana* is a nonpathogen and no therapy is recommended, it is still important to differentiate it from other amebae, some of which are pathogenic. Because these organisms are small, the definitive diagnosis of *E. nana* may have to be made on the basis of the permanent stained smear (Table 21.3). If the original fixation is good, the cysts may be identified on the basis of the wet preparation such as the concentration sediment or flotation surface film. The four nuclear karyosomes appear very refractile in the wet preparation.

Epidemiology and Prevention

As with the other organisms that have been discussed, the mode of transmission is ingestion of the mature cysts in contaminated food or water. The cysts of *E. nana* tend to be less resistant to desiccation than do those of *E. coli*. This organism is also found in the same areas of the world as are the other amebae, that is, in warm, moist climates and in other areas where there is a low standard of personal hygiene and poor sanitary conditions. Prevention would depend on improved personal hygiene and an overall upgrading of sanitary conditions and waste disposal.

Iodamoeba bütschlii

Iodamoeba bütschlii, one of the nonpathogenic amebae, is also worldwide in distribution and was identified in the early 1900s. The original species name of "williamsi" probably referred to atypical cysts of *E. coli*. Although it is found in areas where the other amebae have been recovered, its incidence is not as high as that of *E. coli* or *E. nana*. One of the most striking morphological features of this organism is the large glycogen vacuole which appears in the cyst and readily stains with iodine on a wet preparation smear.

Life Cycle and Morphology

The stages within the life cycle are exactly the same as those seen with *E. nana*. The trophozoite normally varies from 8 to 20 μm and is described as having fairly active motility in a fresh stool preparation (Table 21.1). The cytoplasm is rather granular, containing numerous vacuoles with ingested debris and bacteria. In general, the vacuolated cytoplasm is more obvious than that seen in *E. nana* trophozoites. The nucleus has a large karyosome, which can be either centrally located or eccentric (Fig. 21.12 and 21.14). On the permanent stained smear, the nucleus may appear to have a halo. Chromatin granules fan out around the karyosome. If the granules are on one side, the nucleus may appear to have the "basket nucleus" arrangement of chromatin, which is often found in the cyst stage. The trophozoites of *I. bütschlii* and *E. nana* may be very similar and difficult to differentiate at the species level, even on the permanent stained smear. Both organisms are considered to be nonpathogens, and *E. nana* is recovered in clinical specimens much more frequently than *I. bütschlii*.

The cysts rarely have a classic round appearance but tend to be somewhat oval (Table 21.2). The glycogen vacuole is so large that occasionally the cyst collapses on itself. Since there is no nuclear multiplication in the cyst form, the mature cyst contains only a single nucleus. The cysts measure approximately 5 to 20 μm and are rarely confused with those of other amebae (Fig. 21.12 and 21.14).

Diagnosis

Although the cyst can often be identified from the wet smear preparation, particularly if the vacuole is stained with iodine, the trophozoites may be difficult to detect and identify without the permanent stained smear (Table 21.3). Although these amebae are nonpathogens, they are always re-

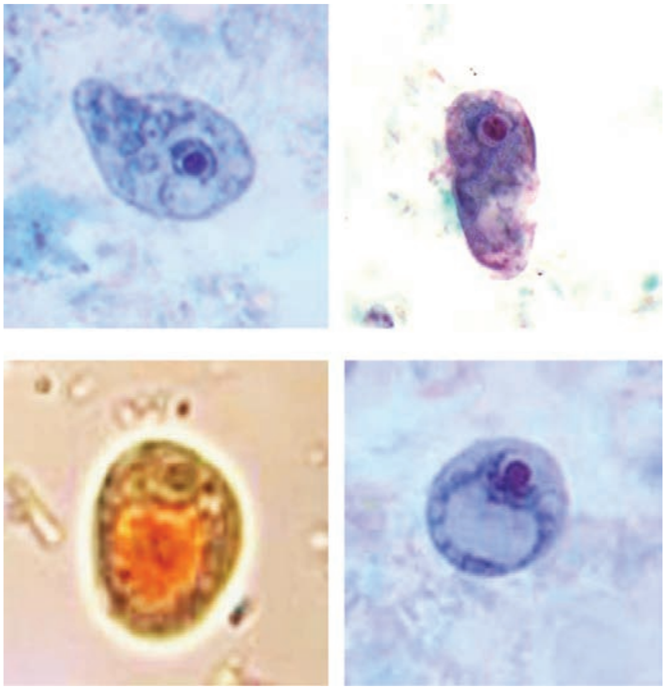


Figure 21.14 (Upper, left and right) Trophozoites of *Iodamoeba bütschlii* (note the very large karyosome within the nucleus). (Lower, left) Cyst of *I. bütschlii* wet mount stained with iodine (note the large glycogen vacuole—stains brownish with iodine); (right) *I. bütschlii* cyst on trichrome-stained permanent stain (also note the large vacuole—appears clear on permanent stain). doi:10.1128/9781555819002.ch21.f14

ported. Their presence in the intestine indicates the patient has ingested something contaminated with fecal material containing the cysts. This is important information for the physician, since pathogens are also acquired the same way.

Epidemiology and Prevention

The mode of transmission is ingestion of infective cysts in contaminated food or water. Prevention would depend on improved personal hygiene and sanitary conditions.

Blastocystis spp.

It is estimated that over 1 billion humans worldwide could be colonized with *Blastocystis* spp. *Blastocystis* sp. (formerly *Blastocystis hominis*), an inhabitant of the human intestinal tract, was first described in 1912. Over the years, this organism been classified as an organism related to *Blastomyces* spp., the cyst form of a flagellate, a yeast of the genus *Schizosaccharomyces*, and a member of the stramenopiles (examples are brown algae such as kelp, diatoms, slime nets, and water molds). However, currently this organism is classified as an unusual anaerobic, single-celled stramenopile (55–58). Although this classification is acceptable at the molecular level, there are discrepancies in morphology between *Blastocystis* and other stramenopiles. Stramenopiles have flagella surrounded by lateral hair-like mastigonemes, which are absent in *Blastocystis*. Based on these issues, revisions have been proposed for the current five-kingdom classification; *Blastocystis* would be placed in a separate sixth kingdom named “Chromista” (class Blastocystea, subphylum Opalinata, infrakingdom Heterokonta, subkingdom Chromobiota, kingdom Chromista [57]).

Blastocystis comprises a multitude of subtypes (species) many of which have been identified only recently; molecular epidemiological studies have revealed a significant difference in the distribution of subtypes across host species and geographical regions. Worldwide, *Blastocystis* is very commonly found in the stools of individuals with and without intestinal symptoms. Following the introduction of molecular methods, the prevalence and intrageneric diversity of *Blastocystis* are becoming better defined. Confirmation of the existence of these species and determination of their pathogenic status may also explain why some patients are asymptomatic and some have clinical symptoms.

Subtypes

Based on small subunit rDNA analysis, *Blastocystis* comprises at least 17 lineages (species); the so-called subtypes (STs) ST1 to ST4 account for probably more than 90% of human carriage, while the remaining approximately 10% are colonized by isolates belonging to ST5 to ST9. All of these STs, apart from ST9, have also been found in various nonhuman hosts (56).

Based on information from multiple studies from various countries, no definitive link has been confirmed between STs and clinical phenotypes. However, at least two main cofactors may complicate the issue. Significant variation in the distribution of STs is seen across geographic ranges; in some areas certain STs are quite common, while in other areas, they may be totally absent. The second cofactor involves the use of different methodologies for subtyping; this also impacts results from ST survey data. The two methods currently in use are screening fecal samples by PCR using either (i) ST-specific primers (sequence-tagged site primers) or (ii) genus-specific primers with subsequent sequencing for ST identification (one method is barcoding covering the 600 5′-most bases of the small subunit rDNA). Both approaches have advantages and disadvantages; see reference 56 for additional information.

Life Cycle and Morphology

Blastocystis reproduces primarily by binary fission and has a membrane-bound central body (previously called a vacuole) that takes up 90% of the cell and plays a role in the programmed cell death of the organism (Fig. 21.15 to

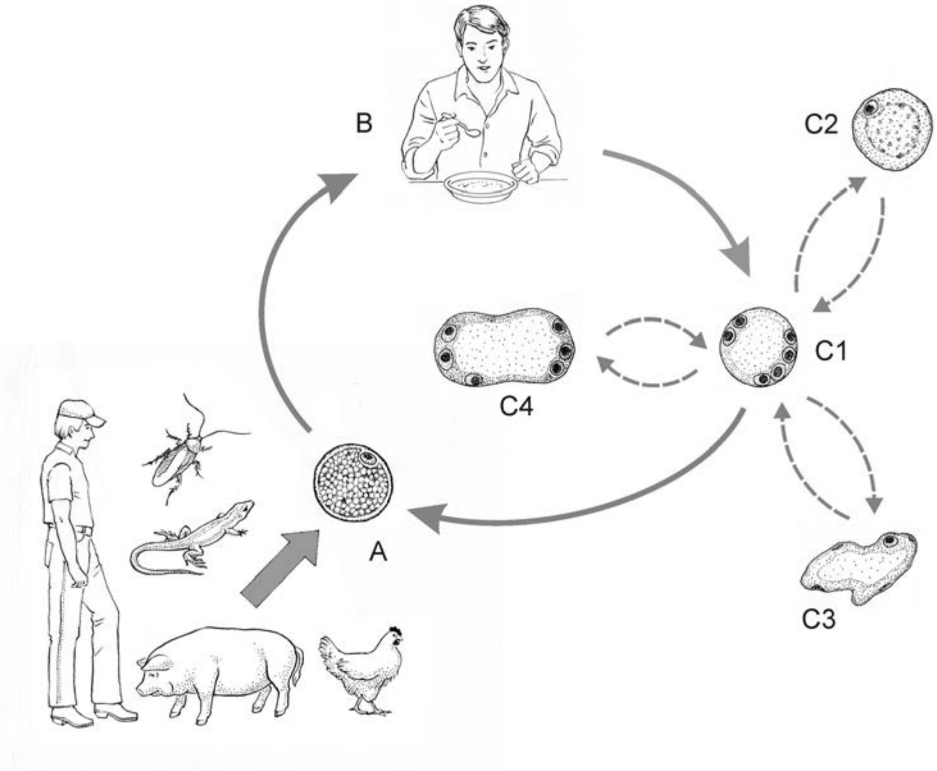


Figure 21.15 Life cycle of *Blastocystis* spp. (A) Cyst forms are excreted in the feces of infected hosts. (B) Humans acquire infection by consumption of food or water contaminated with feces containing cysts. (C) Excystation occurs in the large intestine to release the vacuolar form. The vacuolar form (C1) can transform into the granular form (C2) or the ameboid form (C3) and vice versa. The vacuolar form multiplies by binary fission (C4) (other modes of reproduction such as plasmotomy and budding can also occur). The vacuolar form undergoes encystation in the lumen of the large intestine to produce the cyst form, which is shed in the feces. (Adapted with permission from references 55 and 59.)

doi:10.1128/9781555819002.ch21.f15

21.17; Table 21.7). Budding and plasmotomy are also recognized forms of reproduction. Other structures with unknown function are not yet defined. The organism is strictly anaerobic, normally requires bacteria for growth, and is capable of ingesting bacteria and other debris. The generation time of the organism observed *in vitro* in axenic media is 17 to 22 h. In cultures on xenic media followed by axenization the generation time is shorter, ranging 7 to 12 h. Generation time is also found to vary among different subtypes.

When ingested, the cysts develop into vegetative forms. The cyst form undergoes excystation in the large intestine to liberate the vacuolar form. The vacuolar forms can transform into any of the other forms. Frequent observations of the ameboid, avacuolar, and multivacuolar forms in diarrhea suggest these forms might play a role in the pathogenesis. The vacuolar forms encyst in the intestinal lumen to form cysts which are shed in the feces for further transmission (59).

Organism Types. Recognized morphologic types include the vacuolar, granular, ameboid, avacuolar, and multivacuolar forms. The classic form (vacuolar form) that is usually seen in the human stool specimen varies tremendously in size, from 6 to 63 μm (commonly 5 to 15 μm) in humans, and is characterized by a large central body, which is involved with carbohydrate and lipid storage (visually like a large vacuole) (Fig. 21.16, 21.17; Table 21.7). The granular form resembles the vacuolar form; however, there are granules in the central body and the cytoplasm. The three types of granules are metabolic, reproductory, and lipid. The granular forms range from 15 to 25 μm. The more amebic form (ameboid form) is occasionally seen in diarrheal fluid but may be extremely difficult to recognize. Although this form has one or two pseudopodia, the ameboid forms are nonmotile. Due to their size and morphology, they mimic neutrophils and macrophages; thus, they are easily missed in routine microscopy examinations. Generally, *Blastocystis* is identified on the basis of the more typical round form with the central body. The avacuolar and the multivacuolar forms are smaller, measuring 5 to 8 μm in diameter, and extreme size variations do not occur. The avacuolar form has no central body, while the multivacuolar forms have multiple small vacuoles of different sizes interconnected to each other or lying discrete in the cytoplasm. They are usually uninucleate but occasionally have two nuclei. In the past, these two forms were considered intermediates during transformation of the common vegetative forms; however, recent observations suggest that the avacuolar and the multivacuolar forms are the predominant forms *in vivo* and are not recognized during microscopic examination.

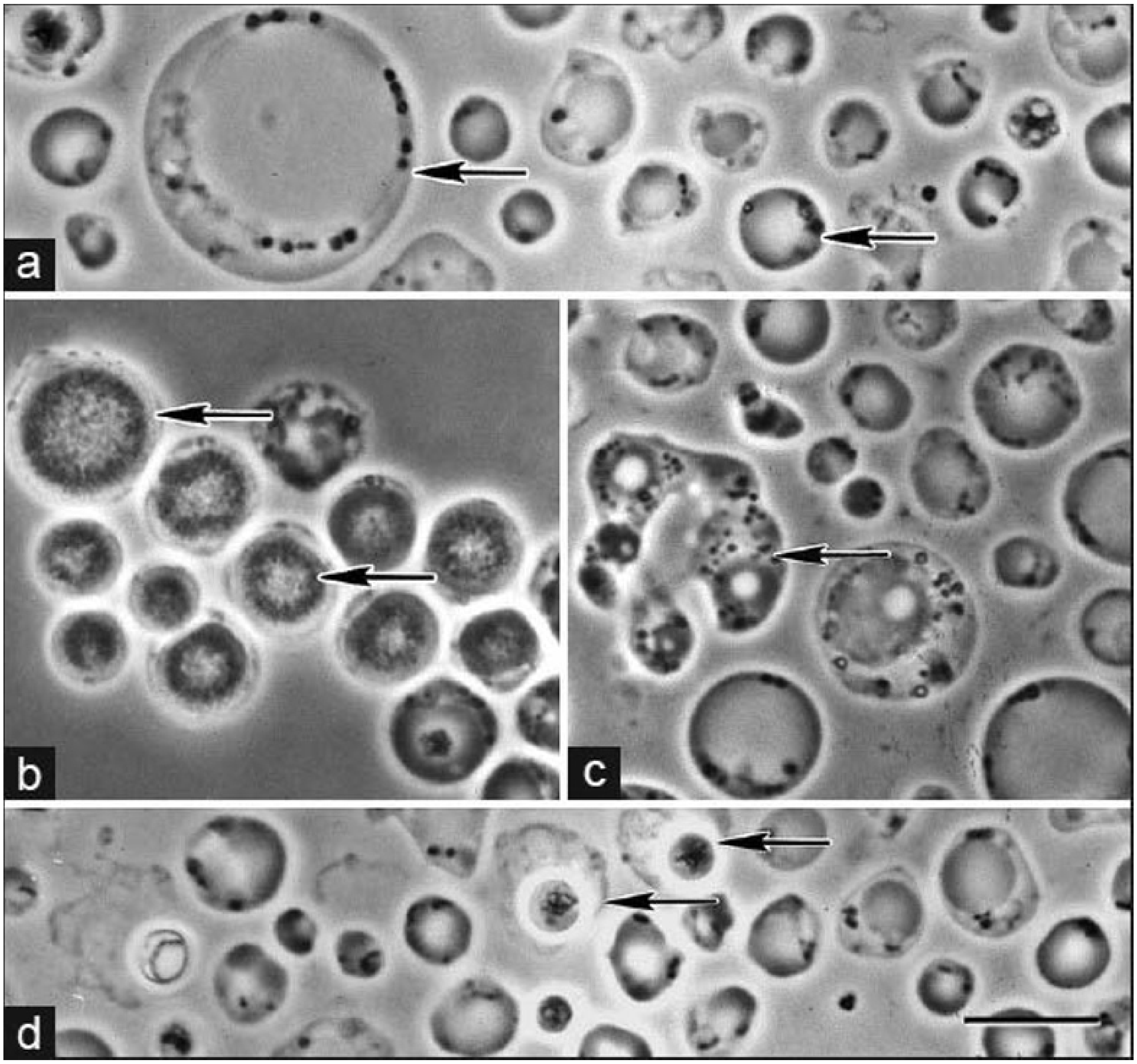


Figure 21.16 Various forms of *Blastocystis*. (a) Vacuolar forms of *Blastocystis* having a large centrally placed vacuole showing extensive variation in size (arrows). (b) Granular forms with distinct granules filling the central body. (c) Ameboid form with characteristic pseudopodia. (d) Cyst forms. Note the smaller size and the characteristic refractile cyst wall surrounded by loose irregular outer coat. Bar, 10 µm. (Reproduced from reference 58.) doi:10.1128/9781555819002.ch21.f16

Both thin- and thick-walled cysts have been confirmed and usually measure 3 to 6 µm. It is suggested that the thin-walled cysts are autoinfectious, leading to multiplication of the organism in the intestinal tract; however, this information appears to be inconclusive. The thick-walled cysts are probably responsible for external transmission via the fecal-oral route, particularly since the cyst wall protects the organism during adverse conditions. The cysts remain viable for up to 1 month at 25°C even on exposure to air. This type of life cycle, or something similar, might explain the high percentage of positive carriers in many studies, showing that the percentage of patients infected with *Blastocystis* is often much higher than the percentage of those infected with other intestinal protozoa (60).

Clinical Disease

When *B. hominis* is present in the absence of other pathogenic parasites, bacteria, or viruses, it may be the cause of diarrhea, cramps, nausea, fever, vomiting, and abdominal pain and may require therapy. In one recent study of patients with irritable bowel syndrome, there was a set of patients in whom the presence of *Blastocystis* did not appear to be incidental (61). Possible relationships between *Blastocystis* and intestinal obstruction and perhaps even

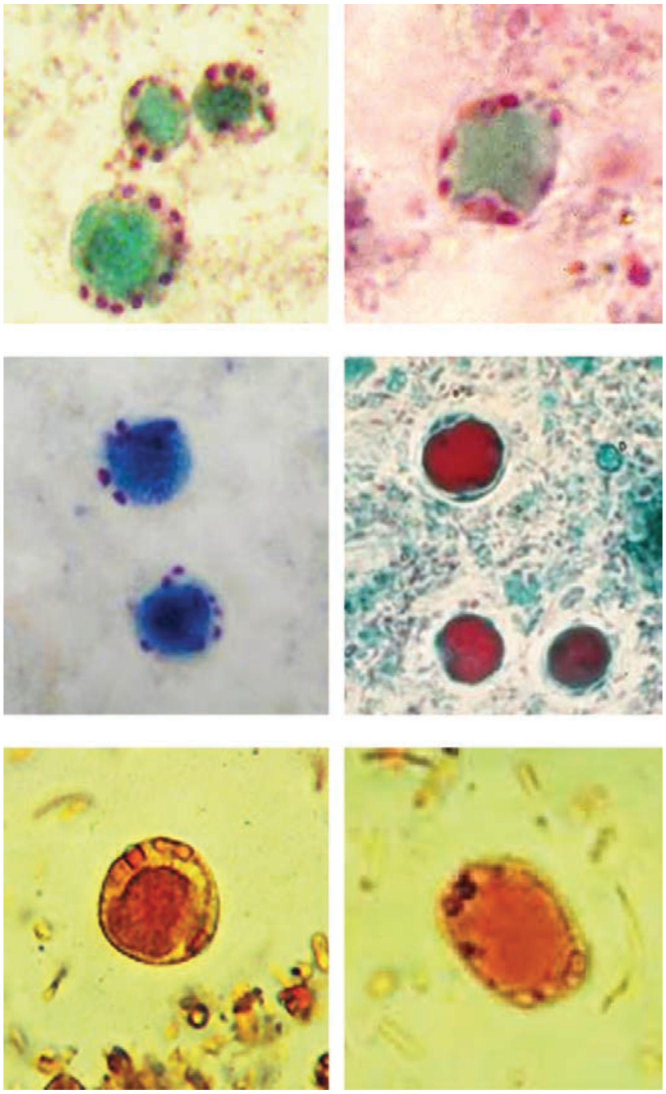


Figure 21.17 (Top, middle) Stained central-body forms of *Blastocystis* spp.; note the multiple nuclei around the edges, outside the central-body area. (Bottom) *Blastocystis* central-body forms in iodine wet mounts; note dividing form in right image (binary fission) and the large central "vacuole."
doi:10.1128/9781555819002.ch21.f17

infective arthritis have been suggested. In patients with other underlying conditions, the symptoms may be more pronounced. The incidence of this organism appears to be higher than suspected in stools submitted for parasite examination. For symptomatic patients in whom no other etiologic agent has been identified, *Blastocystis* should certainly be considered a possible candidate. Other studies suggest that when a symptomatic *Blastocystis* infection responds to therapy, the improvement probably represents elimination of some other undetected pathogenic organism (*E. histolytica*, *G. lamblia*, or *D. fragilis*) (62). Data from other geographic areas indicate that although it is commonly seen in stool samples, *Blastocystis* is thought to be nonpathogenic. Although for a number of years the true role of this organism in terms of colonization or disease was still somewhat controversial, it is now generally considered a causative agent of intestinal disease.

In a study of 1,216 adults, including immunocompromised patients, institutionalized psychiatric or elderly subjects, immigrants from developing countries, travelers to developing tropical countries, and controls, the results showed a high prevalence of parasites in all the risk groups studied, and *Blastocystis* was the parasite most frequently detected in each studied group. *Blastocystis* showed a significant correlation with gastrointestinal symptoms only when detected in the group including subjects with a severe immunodepression (63).

Intestinal Disease. Disease ranges from asymptomatic colonization to severe symptoms. There may be vague abdominal complaints such as pain, bloating, and flatulence. Generally the diarrhea is mild in the immunocompetent patient and more severe and chronic in those who are immunocompromised. There may also be symptoms of irritable bowel syndrome.

Extraintestinal Disease. The following symptoms and conditions have been seen in extraintestinal infections. Often these patients have been found to have other underlying diseases or are immunocompromised. Acute or chronic urticaria has been seen, and palmoplantar pruritus has also been reported (64, 65). Chronic angioedema has been associated with *Blastocystis* infection (66). In another study, a relationship between *Blastocystis* infection and iron deficiency anemia was confirmed; however, there was no correlation found between the genotype and the occurrence of iron deficiency anemia (67). It is also highly recommended that *Blastocystis* be considered in ulcerative colitis patients with refractory symptoms (68).

Diagnosis

Routine Diagnosis. Routine stool examinations are very effective in recovering and identifying *Blastocystis*, although the permanent stained smear is the procedure of choice since the examination of wet preparations may not easily reveal the organism. If the fresh stool is rinsed in water before fixation (for the concentration method), *Blastocystis* organisms, other than the cysts, are destroyed, thus possibly yielding a false-negative report (32). In many areas of the world, *Blastocystis* is the most common parasite found in stool specimens. The characteristic form that is usually seen in human fecal

TABLE 21.7 Key features of *Blastocystis*[a]

Organism form	Size (µm) (most typical)	Central vacuole (membrane-bound body), "central-body form"	No. of nuclei (most typical)	Sites where found	Description
Vacuolar	3–120; 4–63 human (5–15)	Present	1–4 (1–2)	Feces, culture	Central vacuole fills 70–90% of cell; giant cells with multiple nuclei occasionally seen; cytoplasm and organelles limited to thin peripheral rim (may be difficult to see in wet preparations); most common form seen in microscopy of fecal specimens
Granular	15–80 (15–25)	Present	1–4	Feces, culture	Granules within central vacuole
Multivacuolar	5–8	Present or absent	1–2 (1)	Feces, culture	Rarely seen
Avacuolar	~5	Absent	1–2 (1)	Intestine, feces	Rarely seen in feces; very large nuclei
Ameboid	~10	Present (single large vacuole or multiple small ones) or absent	1–2	Feces, culture	Rarely reported; no general consensus on morphology; resemble neutrophils or macrophages; pseudopods may be seen, but these stages are not motile; more often seen in feces of symptomatic patients
Cyst	3–10 (3–6)	Absent	1–4 (2)	Feces, culture	Rarely seen in culture; cyst wall present (varies in thickness); may be covered by a fibrillar layer; oval or round shape; thick, multilayered cyst wall; might lose surface coat and appear as "naked" cysts; viable up to 1 month at 25°C even on exposure to air; transmissible infective forms; common form seen in stored fresh stool specimens

[a] Adapted from references 53–60.

specimens varies in size from 6 to 40 µm and contains a large central body resembling a vacuole, which may be involved with carbohydrate and lipid storage. The amebic form can occasionally be seen in diarrheal fluid but may be difficult to recognize (69). The organisms should be quantitated on the report form, i.e., as rare, few, moderate, or many. It is also important to remember that other possible pathogens should be adequately ruled out before a patient is treated for *Blastocystis*.

Antibody Detection. Both ELISA and fluorescent-antibody tests have been developed for detection of serum antibody to *B. hominis* infections (44, 70). By using ELISA and a threshold dilution of 1/50, 27 of 30 serum samples (28 patients with no protozoan parasites other than *Blastocystis*) were positive at 1/50 or higher; 42 control blood bank serum samples were all negative at 1/50 (44). A strong antibody response is consistent with the ability of this organism to cause symptoms. Also, demonstration of serum antibody production both during and after *Blastocystis* symptomatic disease provides immunological evidence for the pathogenic role for this protozoan, although it may take 2 years or more with chronic infections before a serologic response is detected (44).

Molecular Testing. Using advanced microscopy and sequence-confirmed PCR, a high prevalence of *Blastocystis* infection was found. Most cases were caused by subtypes ST1, ST2, ST3, and ST4. A significantly higher prevalence was found among patients with a history of recent travel to tropical countries (23). A quantitative TaqMan 5′ nuclease real-time PCR (TaqMan PCR) including an internal process control was developed for the detection of *Blastocystis* and shown to be applicable to genomic DNAs extracted directly from feces. This approach was found to be more sensitive than results obtained using xenic *in vitro* culture (5).

KEY POINTS—LABORATORY DIAGNOSIS

Blastocystis spp.

1. Although the intestinal protozoa are not routinely quantitated on the report, *Blastocystis* should be quantitated (rare, few, moderate, many).
2. A minimum of three ova and parasite examinations (including the permanent stained smear) and/or some of the immunoassays should be performed to ensure that no other protozoa are found before attributing symptoms to *Blastocystis*.
3. Other possible microbial agents should also be considered.
4. Remember that there is still controversy regarding quantitation of these organisms on the laboratory report form. Although there was thought to be correlation between numbers of organisms present and patient symptoms, some physicians have seen symptomatic patients in whom even small numbers of *Blastocystis* are present, apparently with no other causative agents present in the stool specimen(s). For this reason, quantitation of the organisms is still recommended. However, it is important to communicate with your physician clients so that they know how you are reporting and/or quantitating this organism. The approach must be determined by each laboratory after discussions with physician clients.

Treatment

Although there is not a great deal of clinical evidence, there have been studies on the *in vitro* susceptibility of *Blastocystis* to numerous drugs. At present, metronidazole (Flagyl) appears to be the most appropriate drug, ranging from 250 to 750 mg three times a day for 10 days to 1.5 mg/day for 10 days, or used in combination with other drugs such as paromomycin or cotrimoxazole (trimethoprim-sulfamethoxazole) (56). In cases of treatment failure, cotrimoxazole and nitazoxanide may be considered as second-choice drugs. Treatment should be considered if diarrhea is persistent and no other pathogen apart from *Blastocystis* is identified in fecal specimens. The development of a new drug sensitivity assay may improve our ability to scientifically evaluate the activities of various drugs against this organism. Because this organism is so widespread throughout the world, it is always going to be somewhat difficult to differentiate between treatment failure and reinfection.

Epidemiology and Prevention

From present information, it appears that *Blastocystis* is transmitted via the fecal-oral route through contaminated food or water. Although other possible modes of transmission are not defined, the incidence and apparent worldwide distribution indicate the traditional route of infection. Studies suggest the existence of numerous zoonotic isolates, with frequent animal-to-human and human-to-animal transmissions, and of a large potential reservoir in animals for infections in humans. Prevention would probably involve improved personal hygiene and sanitary conditions (57, 58). When genotypic results from animal isolates were compared with the diversity of genotypes of human *Blastocystis* spp. isolates, the human isolates were defined as the same as the subtypes of the pet isolates, as well as the tap water isolates. Thus, the possibility of zoonotic transmission appears to be very likely (12).

Very specific prevention measures for all of the intestinal protozoa can be seen in Table 21.8. These recommendations are relevant for all organisms that can be acquired through the ingestion of food or water contaminated with fecal material.

TABLE 21.8 Measures for the prevention of infection with intestinal protozoa

1. Wash hands with soap and water after using the toilet and before handling food.
2. Avoid water or raw food that may be contaminated.
3. Wash and peel all raw vegetables and fruits before eating.
4. When traveling in countries where the water supply may be unsafe, avoid drinking unboiled tap water and avoid uncooked foods washed with unboiled tap water. Bottled or canned carbonated beverages, seltzers, pasteurized fruit drinks, and steaming hot coffee and tea are safe to drink.
5. If you work in a child care center where you change diapers, be sure to wash your hands thoroughly with plenty of soap and warm water after every diaper change, even if you wear gloves.

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22

Intestinal Protozoa: Flagellates and Ciliates

- *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*)
- *Dientamoeba fragilis*
- *Pentatrichomonas hominis* (*Trichomonas hominis*)
- *Trichomonas tenax*
- *Chilomastix mesnili*
- *Enteromonas hominis*
- *Retortamonas intestinalis*
- *Balantidium coli*

Giardia lamblia (*G. duodenalis, G. intestinalis*)

The flagellate *Giardia lamblia* (syn. *Giardia duodenalis*, *Giardia intestinalis*) was first discovered by Leeuwenhoek in 1681 in his own stool specimens but was not described until 1859 by Lambl. The organism was named after Professor A. Giard of Paris and Dr. F. Lambl of Prague (1). *G. lamblia* is worldwide in distribution and apparently is more prevalent in children than in adults and more common in warm climates than in cool ones. It is the most commonly diagnosed flagellate in the intestinal tract, and it may be the most commonly diagnosed intestinal protozoan in some areas of the world. Although a number of papers on the nomenclature of *Giardia duodenalis* replacing *G. lamblia* or *G. intestinalis* have been published, apparently this change has not been formally recognized by the International Code of Zoological Nomenclature. Therefore, we will maintain the name *G. lamblia* for this edition.

Although various criteria, including host specificity, body dimensions, and variations in structure, have been used to differentiate species of *Giardia* (*G. duodenalis*, *G. intestinalis*), there is still considerable debate over the appropriate classification and nomenclature regarding this group of organisms. On the basis of work by Filice related to structural variations, three groups have been proposed: amphibian *Giardia* spp. (represented by *G. agilis*), the muris group from rodents and birds (represented by *G. muris*), and the intestinalis group from a variety of mammals (including humans), birds, and reptiles (represented by *G. duodenalis*) (2). During the 1980s, the name *G. duodenalis* was supported and in the 1990s *G. intestinalis* was supported by various investigators. Despite disagreement concerning the various species names, all three continue to be used to describe this organism. Meyer prefers to use *Giardia duodenalis*, which is often followed by the name of the animal from which the organism was obtained (3). Within the United States, the term *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) has been commonly used for many years and will continue to be used throughout this text to refer to those organisms found in humans and other mammals; this designation will, we hope, eliminate any confusion, since the majority of health workers are used to this name and

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TABLE 22.1 *Giardia* species[a]

Species	Hosts	Morphology: Light microscopy	Morphology: Electron microscopy	Molecular data
G. agilis	Amphibians	Long, slender; long teardrop-shaped median body	Focal contacts by the lateral crest of the ventral disk, the ventrolateral flange, the lateral shield, and by numerous microvillus-like appendages found along the lateral border of the trophozoite	NA[b]
G. ardeae	Herons	Pear shaped; 1 or 2 transverse, claw-hammer-shaped median bodies	Ventral disk and caudal flagellum similar to *G. muris*	Closer to *G. lamblia* than to *G. muris*
G. lamblia[c]	Humans and many other mammals, birds, reptiles	Pear shaped; 1 or 2 transverse, claw-hammer-shaped median bodies; sucking disk shorter than half the body length	Nuclei have a defined position, and fibrils perform an anchoring system; median bodies vary in number, shape, and position, are found in mitotic and interphasic trophozoites, are present in about 80% of the cells, and are not completely free in the cells	Clade with multiple genotypes
G. microti	Voles and muskrats	Pear shaped; 1 or 2 transverse, claw-hammer-shaped median bodies	Cysts contain 2 trophozoites with mature ventral disks	Similar to *G. lamblia* genotypes
G. muris	Rodents, birds, reptiles	Short and rounded; small round median body	Cysts contain 1 trophozoite; divides on excystation	Distant from *G. lamblia*
G. psittaci	Psittacine birds	Pear shaped; 1 or 2 transverse, claw-hammer-shaped median bodies	Incomplete ventrolateral flange, no marginal groove bordering adhesive disk	Not more diverse genetically than other *Giardia*

[a] Adapted from references 2 and 12.

[b] NA, not available.

[c] Although a number of papers on the nomenclature of *Giardia duodenalis* replacing *G. lamblia* or *G. intestinalis* have been published, apparently this change has not been formally recognized by the International Code of Zoological Nomenclature. Therefore, we will maintain the name *G. lamblia* for this edition.

continue to report the presence of the organism by using the term "*Giardia lamblia*" (Table 22.1).

Molecular classification methods have been helpful in understanding the pathogenesis and epidemiology of human and animal *Giardia* infections. These studies have confirmed the division of *Giardia* human isolates into two major genotypes (Table 22.2). Adam has proposed that genotype A be accepted as the designation for Nash group 1 (A-1), Nash group 2 (A-2), assemblage A, and the Polish isolates. Genotype B would designate Nash group 3, assemblage B, and the isolates from Belgium. He feels it may also be appropriate to use a similar designation for assemblages C through G, since these also reflect both genetic and host differences (2, 4). Subassemblages AI, AII, BIII, and BIV are commonly identified in human cases (5). Another assemblage, H, has been identified in marine vertebrates (6). To date, eight main assemblages of *Giardia* have been described, but only A and B genetic groups are known to infect humans.

Life Cycle and Morphology

The life cycle is seen in Fig. 22.1. Both the trophozoite and the cyst are included in the life cycle of *G. lamblia*. Trophozoites divide by means of longitudinal binary fission, producing two daughter trophozoites. The most common location of the organisms is in the crypts within the duodenum. The trophozoites are the intestinal dwelling stage and attach to the epithelium of the host villi by means of the ventral disk. The attachment is substantial and results in disk "impression prints" when the organism detaches from the surface of the epithelium. Trophozoites may remain attached or detach from the mucosal surface. Since the epithelial surface sloughs off the tip of the villous every 72 h, apparently the trophozoites detach at that time.

There are several hypotheses on possible mechanisms for attachment by the ventral disk; these include microtubule mediation, hydrodynamic action, contractile protein activity, and the interaction of lectins with surface-bound sugars. Because there is conflicting information, the attachment process probably depends on multiple mechanisms. Attachment to enterocyte-like differentiated Caco-2 cells is primarily by cytoskeletal mechanisms, which can be inhibited by interfering with contractile filaments and microtubules; attachment by mannose-binding lectin also seems to mediate binding (7). In addition to the ventral disc, *Giardia* trophozoites have a complex three-dimensional microtubule cytoskeleton, including eight flagella (anterior, caudal, posterior-lateral, and ventral) with cytoplasmic and

TABLE 22.2 *Giardia* genotypes[a]

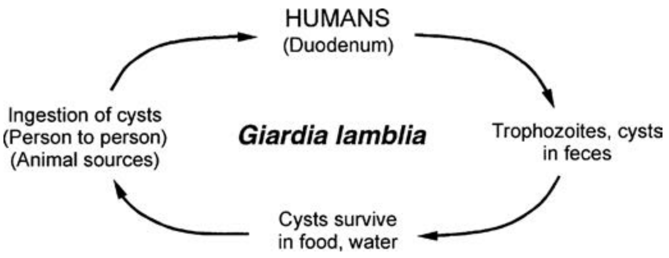
Adam designation	Nash group	Mayrhofer assemblage	Subassemblages in humans	Origin	Hosts
Genotype A-1	1	A (group 1)	AI	Poland	**Human**, beaver, cat, lemur, sheep, calf, dog, chinchilla, alpaca, horse, pig, cow, water buffalo, gerbil, moose, Mediterranean mussels, squirrel monkey, roe deer
Genotype A-2	2	A (group 2)	AII		**Human specific,** beaver, monkey
	3	A (group 3)			Red deer, cat
Genotype B	3	B (group 3)	BIII	Belgium	**Human,** beaver, guinea pig, dog, monkey, cat, deer, coyote, rabbit
	3	B (group 4)	BIV	Belgium	**Human specific**
		C			Dog, coyote
		D			Dog, cat, coyote
		E (or A, livestock)			Cow, sheep, alpaca, goat, pig, gerbil
		F			Cat, pig
		G			Rodents
		H			Seal, gull

[a] Adapted from references 2, 4, 5, and 6.

membrane-bound regions, the median body, the funis, and the supernumerary microtubules (8).

The development of viable cysts by *Giardia* is essential for its survival in the environment and for spreading the infection through contaminated food and water. For reasons which are not fully known, cyst formation takes place as the organisms move down through the jejunum after exposure to biliary secretions. However, it has recently been reported that DNA topoisomerase II is involved in regulation of cyst wall protein genes and differentiation (9). It also appears that glucosylceramide transferase activity is critical for encystation and viable cyst production in *Giardia* (10). The trophozoites retract the flagella into the axonemes, the cytoplasm becomes condensed, and the cyst wall is secreted. During this process, encystation-specific vesicles are developed that transport cyst wall proteins to the plasma membrane of the trophozoite before the production of the protective cyst wall (10). As the cyst matures, the internal structures are doubled, so that when excystation occurs the cytoplasm divides, thus producing two trophozoites. Excystation would normally occur in the duodenum or appropriate culture medium (Fig. 22.1) (2).

Figure 22.1 Life cycle of *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*). doi:10.1128/9781555819002.ch22.f1



The process of encystment consists of two morphologically different stages (intracellular and extracellular) and requires approximately 16 h to complete. The extracellular phase is initiated with the appearance of cyst wall antigen on small protrusions of the trophozoite membrane, which becomes enlarged over time to form caplike structures ranging up to 100 nm in diameter. Caplike structures involved with filament growth have been detected over the entire surface of the trophozoite including the adhesive disk and flagella. Encysting cells round up, lose attachment to the substratum, and become enclosed in a layer of filaments. The internal portion includes two trophozoites with four nuclei. If methods could be found to interfere with the development of the filamentous layer of the cyst wall, regulation of viable *Giardia* cyst release from the host could be modified or eliminated (2).

Changes observed in *Giardia* lipids (increased fatty acid unsaturation and the accumulation of storage lipids) are consistent with parasite differentiation into a cyst stage that can survive outside the host at reduced temperatures and with reduced available nutrient resources. *Giardia* not only has the capacity to synthesize isoprenoid lipids *de novo* but also can metabolize fatty acids by the addition of double bonds (2). Studies have also shown that bile acids found in high concentrations in the small intestine enhance trophozoite survival, multiplication, and differentiation into the cyst stage. Results also indicate that cholesterol starvation is necessary and sufficient for the stimulation of *Giardia* encystations *in vitro* and probably in the intestines of mammalian hosts (11).

It has been determined that oxygen uptake rate and metronidazole sensitivity in *Giardia* cysts are greatly reduced compared with those in trophozoites. The resistance to metronidazole may be due to a change in metabolic flux away from the pyruvate ferredoxin oxireductase pathway. However, after the induction of excystation, oxygen uptake increases exponentially during the following 30 min and metronidazole sensitivity returns within 15 min and is fully restored within 30 min (2). Excystation occurs with exposure to the contents of the proximal small intestine; the process is quite rapid. Externally, the flagella emerge through the cyst wall, followed by the entire trophozoite. Findings on several aspects of *Giardia* differentiation can be found in the review by Adam (2). The process is divided into three distinct parts: (i) the stimulus for encystations and the regulation of encystation-specific gene expression, (ii) the synthesis and intracellular transport of cyst wall components, and (iii) the assembly of the extracellular cell wall.

Although *Giardia* has many features of the prokaryotes (anaerobic metabolism, an enzyme that reduces oxygen directly to water, cysteine as the "keeper" of the redox balance, a plasmid, and toxin-like genes), unlike prokaryotes it also has a sophisticated, highly developed cytoskeleton, bounded nuclei, linear chromosomes capped with telomeric repeats, and telomere positional regulation of gene expression (12, 13). Studies have also clarified the pathway of formation of ethanol, one of the major end products of the fermentative metabolism in *Giardia*, which lacks mitochondria. In contrast to most eukaryotes, in which ethanol formation proceeds from pyruvate via acetaldehyde, the *Giardia* pathway starts from acetyl coenzyme A, a more distal product of extended glycolysis (14).

Trophozoite (Table 22.3). The trophozoite is usually described as being teardrop shaped from the front, with the posterior end being pointed (Fig. 22.2 to 22.4). If one examines the trophozoite from the side, it resembles the curved portion of a spoon. The concave portion is the area of the sucking disk (Fig. 22.2). There are four pairs of flagella, two nuclei, two axonemes, and two slightly curved bodies called the median bodies. The trophozoites usually measure 10 to 20 µm in length and 5 to 15 µm in width.

Cyst (Table 22.4). The cysts may be either round or oval, and they contain four nuclei, axonemes, and median bodies (Fig. 22.3 and 22.4). Often, some cysts appear to be shrunk or distorted, and one may see two halos, one around the cyst wall itself and one inside the cyst wall around the shrunken organism. The halo effect around the outside of the cyst is particularly visible on the permanent stained smear. Cysts normally measure 11 to 14 µm in length and 7 to 10 µm in width.

Clinical Disease

From available data, it appears that the incubation time for giardiasis ranges from approximately 12 to 20 days. Because the acute stage usually lasts only a few days, giardiasis may not be recognized as the cause but may mimic acute viral enteritis, bacillary dysentery, bacterial or other food poisoning, acute intestinal amebiasis, or "traveler's diarrhea" (toxigenic *Escherichia coli*). However, the type of diarrhea plus the lack of blood, mucus, and cellular exudate is consistent with giardiasis.

Although the organisms in the crypts of the duodenal mucosa may reach very high densities, they may not cause any pathology. The organisms feed on the mucous secretions and do not penetrate the mucosa as *G. muris* has been shown to do in mice, from which trophozoites have been recovered in the deeper tissues. Although organisms have been seen in biopsy material obtained from inside the intestinal mucosa, others have been seen attached only to the epithelium. For some reason, in symptomatic patients there may be irritation of the mucosal lining, increased mucus secretion, and dehydration (15).

A study by Chen et al. has demonstrated a *G. duodenalis* cysteine-rich surface protein (CRP136) that has 57% homology to the product of the gene encoding the precursor of the sarafotoxins, a group of snake toxins from the burrowing adder that are known to cause symptoms similar to those of humans acutely infected with *Giardia* species (16). Thus, CRP136 represents the first evidence for a potential *Giardia* toxin.

Onset may be accompanied by nausea, anorexia, malaise, low-grade fever, and chills, and there may be a sudden onset of explosive, watery, foul-smelling diarrhea. Other symptoms include epigastric pain, flatulence, and diarrhea with increased amounts of fat and mucus in the stool but no blood (13). Weight loss often accompanies these symptoms. Although there is speculation that the organisms coating the mucosal lining may act to prevent fat absorption, this does not completely explain why the uptake of other substances normally absorbed at other intestinal levels is prevented. During the acute phase of giardiasis, jejunal active-antigen uptake is increased, leading to a delayed recruitment of mucosal and connective tissue mast cells. These changes may play a role in the increased incidence of hypersensitivity reactions and allergic disease associated with *Giardia* infection (17). Severe malabsorption has also been linked with isolated levothyroxine malabsorption, leading to severe hypothyroidism (18) and secondary impairment of pancreatic function (19). In both cases, treatment with metronidazole led to complete remission of symptoms. Occasionally, the gallbladder is also involved, causing gallbladder colic and jaundice. *G. lamblia* has also been identified in bronchoalveolar lavage fluid (20).

TABLE 22.3 Intestinal protozoa: trophozoites of flagellates

Characteristic	Shape and size	Motility	No. of nuclei and visibility	No. of flagella (usually difficult to see)	Other features
Dientamoeba fragilis	Shaped like amebae; 5–15 μm; usual range, 9–12 μm	Usually nonprogressive; pseudopodia are angular, serrated, or broad lobed and almost transparent	Percentage may vary, but 40% of organisms have 1 nucleus and 60% have 2 nuclei; not visible in unstained preparations; no peripheral chromatin; karyosome is composed of a cluster of 4 to 8 granules	No visible flagella	Cytoplasm finely granular and may be vacuolated with ingested bacteria, yeasts, and other debris; may be great variation in size and shape on a single smear
Giardia lamblia (*G. duodenalis, G. intestinalis*)	Pear shaped; length, 10–20 μm; width, 5–15 μm	"Falling-leaf" motility may be difficult to see if organism is in mucus	2; not visible in unstained mounts	4 lateral, 2 ventral, 2 caudal	Sucking disc occupying 1/2–3/4 of ventral surface; pear-shaped front view; spoon-shaped side view
Chilomastix mesnili	Pear shaped; length, 6–24 μm (usual range, 10–15 μm); width, 4–8 μm	Stiff, rotary	1; not visible in unstained mounts	3 anterior, 1 in cytostome	Prominent cytostome extending 1/3–1/2 length of body; spiral groove across ventral surface
Pentatrichomonas hominis (*Trichomonas hominis*)	Pear shaped; length, 5–15 μm (usual range, 7–9 μm); width, 7–10 μm	Jerky, rapid	1; not visible in unstained mounts	3–5 anterior, 1 posterior	Undulating membrane extends length of the body; posterior flagellum extends free beyond end of body
Trichomonas tenax	Pear shaped; length, 5–12 μm (avg, 6.5–7.5 μm); width, 7–9 μm	Jerky, rapid	1; not visible in unstained mounts	4 anterior, 1 posterior	Seen only in preparations from mouth; axostyle (slender rod) protrudes beyond the posterior end and may be visible; posterior flagellum extends only halfway down body and there is no free end
Enteromonas hominis	Oval; length, 4–10 μm (usual range, 8–9 μm); width, 5–6 μm	Jerky	1; not visible in unstained mounts	3 anterior, 1 posterior	One side of body flattened; posterior flagellum extends free posteriorly or laterally
Retortamonas intestinalis	Pear shaped or oval; length, 4–9 μm (usual range, 6–7 μm); width, 3–4 μm	Jerky	1; not visible in unstained mounts	1 anterior, 1 posterior	Prominent cytostome extending approximately 1/2 length of body

The acute phase is often followed by a subacute or chronic phase. Symptoms in these patients include recurrent, brief episodes of loose, foul-smelling stools; there may be increased distention and foul flatus. Between passing the mushy stools, the patient may have normal stools or may be constipated. Abdominal discomfort continues to include marked distention and belching with a rotten-egg taste. Chronic disease must be differentiated from amebiasis; disease caused by other intestinal parasites such as *Dientamoeba fragilis*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora* (*Isospora*) *belli*, and *Strongyloides stercoralis*; and inflammatory bowel disease and irritable colon. On the basis of symptoms such as upper intestinal discomfort, heartburn, and belching, giardiasis must also be differentiated from duodenal ulcer, hiatal hernia, and gallbladder and pancreatic disease.

Ophthalmic examinations were performed on 141 children with active or past giardiasis. Salt-and-pepper retinal changes (with normal electroretinographic findings) were diagnosed in 28 (19.9%) of the children, all of whom were consistently younger than those with normal retinas. Although these findings indicate that asymptomatic, nonprogressive retinal lesions were particularly common in younger children with giardiasis, the risk does not seem to be related to the severity of the infection, its duration, or the use of metronidazole, but may reflect a genetic disposition (21).

Studies document antigenic variation with surface antigen changes during human infections with *Giardia*;

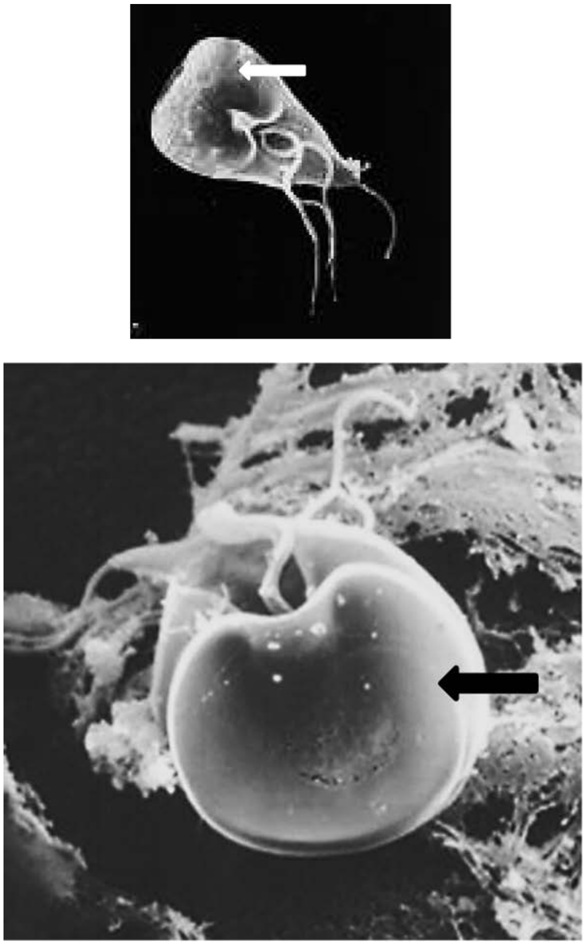


Figure 22.2 *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) trophozoites. Scanning electron micrographs. Note the sucking disk (arrows). (Upper, courtesy of the CDC Public Health Image Library; lower, courtesy of Marietta Voge.)
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although the biological importance of this work is not clear, it suggests that this variation may provide a mechanism for the organism to escape the host immune response (22) and to be responsible for chronic and recurrent infections in susceptible individuals (23). The variant-specific surface proteins are a family of related, highly unusual proteins that cover the surface of the organism; these proteins are resistant to the effects of intestinal proteases, thus allowing the parasites to survive in the protease-rich small intestine. Antigenic variation at the surface membrane of trophozoites occurs frequently; one would suspect that the higher the rate of change, the more likely that a chronic infection would be seen. However, certain surface antigens that allow the organisms to survive better in the intestinal tract may not be immunologically selected. A comprehensive analysis of the superfamily 2 helicases from *Giardia* indicates that variable expression of these helicases is present during antigenic variation and encystation (24).

Although patients with symptomatic giardiasis usually have no underlying abnormality of serum immunoglobulins, a high incidence of giardiasis occurs in patients with immunodeficiency syndromes, particularly in those with common variable hypogammaglobulinemia (25). Giardiasis was the most common cause of diarrhea in these patients and was associated with mild to severe villous atrophy. Successful treatment of giardiasis led to symptomatic cure and improvement in mucosal abnormalities, with the exception of nodular lymphoid hyperplasia.

With the advent of AIDS, there was speculation that *Giardia* might be an important pathogen in this group of individuals. However, clinical findings to date do not seem to confirm this possibility (26). Despite a suppressed immune system in AIDS patients, the immune response to *Giardia* does not seem to be very different from that seen in non-AIDS patients. It does not appear that drugs such as corticosteroids, cyclosporin A, and other immunosuppressive agents of cell-mediated immunity affect the outcome of *Giardia* infections in humans.

Diagnosis

Routine Diagnostic Procedures. Routine stool examinations are normally recommended for the recovery and identification of intestinal protozoa (27). However, in the case of *Giardia*, because the organisms are attached so securely to the mucosa by means of the sucking disk, a series of even five or six stool samples may be examined without recovering the organisms. The organisms also tend to be passed in the stool on a cyclical basis. The Entero-Test capsule can be helpful in recovering the organisms, as can the duodenal aspirate. Although cysts can often be identified on the wet stool preparation, many infections may be missed without the examination of a permanent stained smear (27). If material from the string test (Entero-Test) or mucus from a duodenal aspirate is submitted, it should be examined as a wet preparation for motility; however, motility may be represented by nothing more than a slight flutter of the flagella because the organism is caught up in the mucus. After diagnosis, the rest of the positive material can be preserved as a permanent stain.

Fluoroscopy may reveal hypermotility at the duodenal and jejunal levels, and X rays may reveal mucosal defects. If the cause is *Giardia*, the organisms may be recovered in a series of stool examinations. However, because giardiasis may not produce any symptoms at all, demonstration of the organism in symptomatic patients may not rule out other possibilities such as peptic ulcer, celiac disease of some other etiology, strongyloidiasis, and possibly carcinoma. Refer to Algorithm 22.1 for detailed information.

Antigen Detection. The development of fecal immunoassays for the detection of *Giardia* and/or *Cryptosporidium*

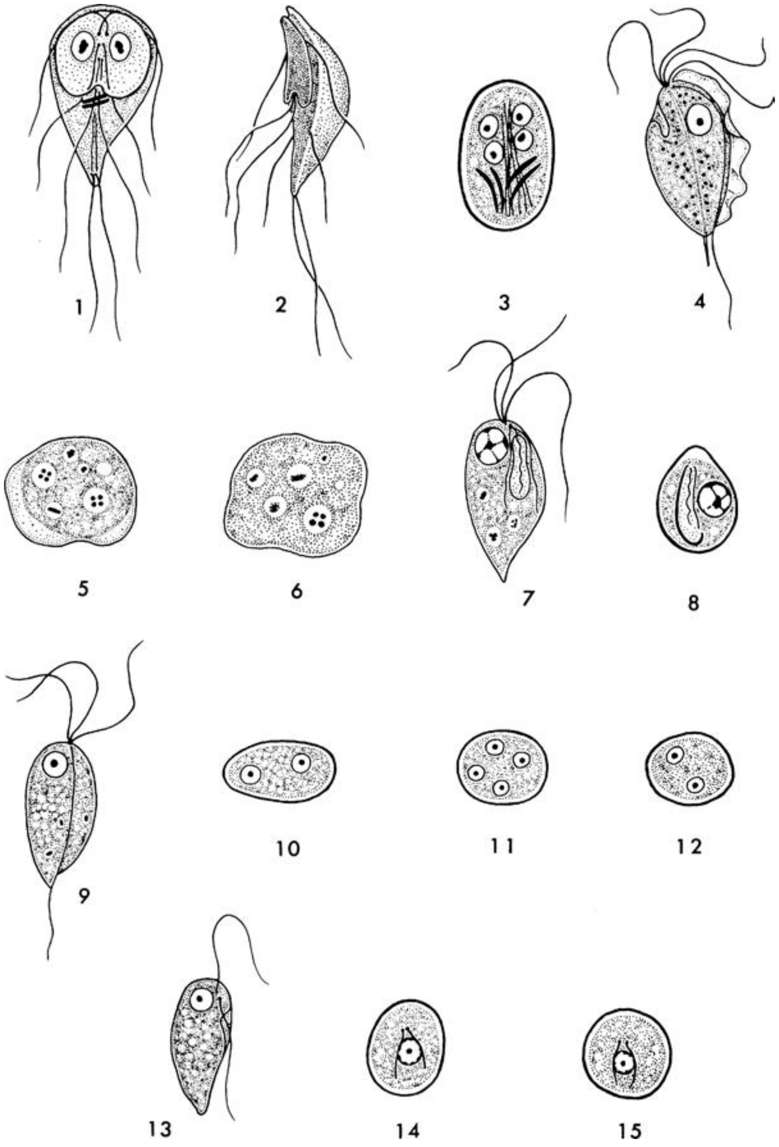

Figure 22.3 (1) Trophozoite of *Giardia lamblia* (front view); (2) trophozoite of *G. lamblia* (side view); (3) cyst of *G. lamblia*; (4) trophozoite of *Pentatrichomonas hominis*; (5) trophozoite of *Dientamoeba fragilis* (two fragmented nuclei); (6) trophozoite of *D. fragilis* (one fragmented nucleus); (7) trophozoite of *Chilomastix mesnili*; (8) cyst of *C. mesnili*; (9) trophozoite of *Enteromonas hominis*; (10–12) cysts of *E. hominis*; (13) trophozoite of *Retortamonas intestinalis*; (14 and 15) cysts of *R. intestinalis*.
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antigen in stool has dramatically improved the sensitivity over that seen with the routine ova and parasite examination and/or modified acid-fast stains (Table 22.5). Procedures involving the enzyme-linked immunosorbent assay (ELISA) have been developed to detect *Giardia* antigen in feces. The ELISA is at least as sensitive as microscopic wet examinations (28–30). Fluorescent methods with monoclonal antibodies have also proven to be extremely sensitive and specific in detecting *G. lamblia* in fecal specimens (31–34). A number of studies have compared the immunoassay products, with excellent and comparable results (28–34). Other products are available as a cartridge format that uses an immunochromatographic strip-based detection system for *G. lamblia* and/or *Cryptosporidium* spp. (35–37). Another cartridge format is used to test for the *Entamoeba histolytica/Entamoeba dispar* group, *Giardia lamblia*, and *Cryptosporidium* spp. This commercially available reagent kit requires fresh or frozen stools, since formalin interferes with the *Entamoeba* reagents (34). Any available antigen detection system should always be reviewed for compatibility with stools submitted in preservatives rather than fresh specimens. There are limitations when using kits that include organisms in the genus *Entamoeba*. However, commercial reagent kits for the detection of *Giardia* and *Cryptosporidium* can be used with formalin-based stool preservatives, the single-vial system (no mercury, PVA, or formalin), as well as fresh or frozen specimens. Many of these cartridge format tests are now available that detect both *Giardia* and *Cryptosporidium*, provide an answer within 10 min, and are equal to or better than other immunoassays in their sensitivity and specificity. Many of these newer methods are being used to test patients suspected of having giardiasis or those who may be involved in an outbreak

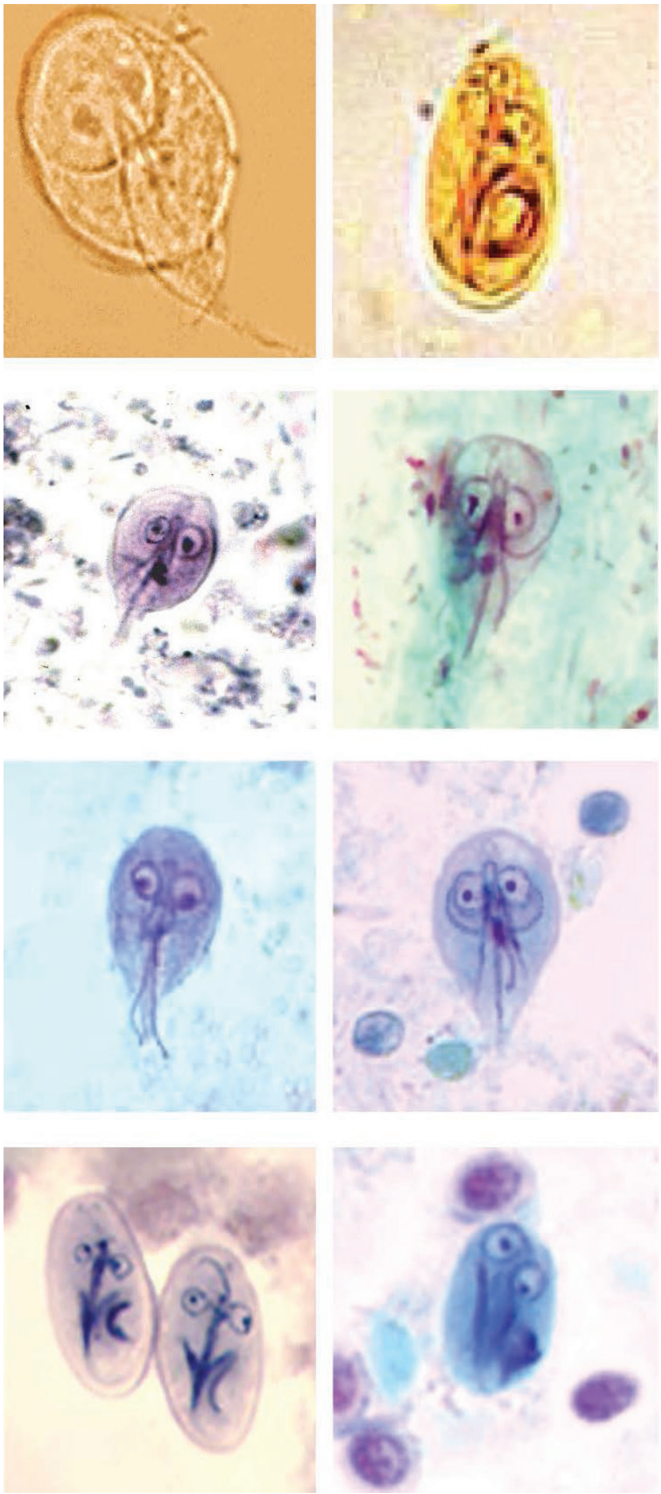


Figure 22.4 *Giardia lamblia* (*G. duodenalis*, *G. intestinalis*) (Top, left) Trophozoite in iodine wet mount; (right) cyst in iodine wet mount. (Row 2, left) Trophozoite stained with iron-hematoxylin. (Right, and both images in row 3) Trophozoites stained with Wheatley's trichrome. (Bottom) Cysts of *Giardia* (left, iron-hematoxylin; right, Wheatley's trichrome).
doi:10.1128/9781555819002.ch22.f4

situation. The detection of antigen in stool or visual identification of organisms by using monoclonal antibody reagents indicates current infection. With the increase in *Giardia* infections and awareness of particular situations such as nursery school settings, these detection assays are rapid and reliable immunodiagnostic procedures. However, it has been well documented that the use of a fecal immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia* (38).

Antibody Detection. Unfortunately, serodiagnostic procedures for giardiasis do not yet fulfill the criteria necessary for wide clinical use, particularly since they may indicate either past or present infection (25). Studies comparing serum antibody responses to giardiasis in symptomatic and asymptomatic patients revealed that antigen recognition by anti-parasite IgM, IgA, IgG1, and IgG3 of these patients, determined by immunoblotting, was heterogeneous and revealed only minor differences in the responses of the two groups (25, 39). In a study investigating the secretory immune response to membrane antigens during giardiasis in humans, saliva samples showed a heterogeneous response to the membrane fraction when they were assayed by immunoblotting. Among the antigens recognized by patient saliva samples, those of 170, 105, 92, 66, 32, 29, and 14 kDa stood out since they were not recognized by saliva samples from healthy individuals. These findings may become more relevant in future studies of protection from or diagnosis of *Giardia* infections (40). Since *Giardia* trophozoites rarely invade the tissues or stimulate the systemic immune response, serodiagnostic assays fail to show differences in serum antibody responses between symptomatic and asymptomatic patients and are not recommended.

Histology. Trophozoites are detectable in the duodenum and proximal jejunum of infected patients. Although there has been some suggestion of mucosal invasion by this parasite, mucosal invasion has generally been found in areas where necrosis or mechanical trauma was present. In reviewing descriptions of general mucosal appearances, there can be a spectrum of change from normal to almost complete villous atrophy, with a greater density of inflammatory infiltrate in the lamina propria when villous atrophy is present. Apparently, patients with giardiasis also have reduced mucosal surface areas compared with controls, and the degree of abnormality correlates with indices of malabsorption. Enumeration of lamina propria plasma cells of different immunoglobulin classes reveals no clear patterns, however, suggesting that *Giardia* infection initially provokes IgM and IgE synthesis, with relative suppression of local IgA production.

Histologic changes in the mucosal architecture in immunodeficient patients with giardiasis also range from

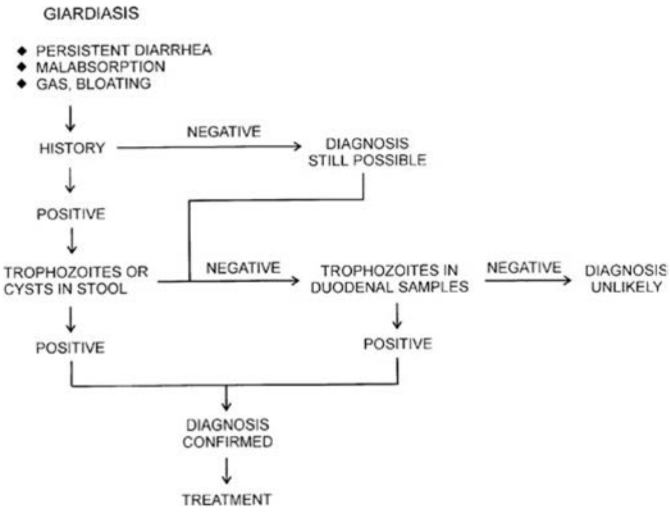
TABLE 22.4 Intestinal protozoa: cysts of flagellates

Species	Size	Shape	No. of nuclei	Other features
Pentatrichomonas hominis (*Trichomonas hominis*), *Trichomonas tenax*	No cyst stage			
Dientamoeba fragilis	~5–8 μm; inner organism about 5 μm; inner, outer cyst walls; very few cysts seen; trophozoites more common	Oval to round	1 or 2	Distinct cyst wall; inner cyst wall irregular, located directly adjacent to encysted parasite; peritrophic space exists between outer cyst wall and encysted parasite. Koch's postulates fulfilled with mice/rats; fecal-oral cycle established; confirmed in human fecal specimens
Giardia lamblia (*G. duodenalis*, *G. intestinalis*)	Length, 8–19 μm (usual range, 11–14 μm); width, 7–10 μm	Oval, ellipsoidal, or may appear round	4; not distinct in unstained preparations; usually located at one end	Longitudinal fibers in cysts may be visible in unstained preparations; deep-staining median bodies usually lie across the longitudinal fibers; there is often shrinkage, and the cytoplasm pulls away from the cyst wall; there may also be a halo effect around the outside of the cyst wall because of shrinkage caused by dehydrating reagents
Chilomastix mesnili	Length, 6–10 μm (usual range, 7–9 μm); width, 4–6 μm	Lemon-shaped with anterior hyaline knob	1; not distinct in unstained preparations	Cytostome with supporting fibrils, usually visible in stained preparation; curved fibril along side of cytostome usually referred to as "shepherd's crook"
Enteromonas hominis	Length, 4–10 μm (usual range, 6–8 μm); width, 4–6 μm	Elongate or oval	1 to 4; usually 2 lying at opposite ends of cyst; not visible in unstained mounts	Resembles *E. nana* cyst; fibrils or flagella usually not seen
Retortamonas intestinalis	Length, 4–9 μm (usual range, 4–7 μm); width, 5 μm	Pear shaped or slightly lemon shaped	1; not visible in unstained mounts	Resembles *Chilomastix* cyst; shadow outline of cytostome with supporting fibrils extends above nucleus; "bird beak" fibril arrangement

mild to severe villous atrophy. Nodular lymphoid hyperplasia has been reported, as well as mixed lesions. Again, the amount of villous damage seems to correlate with the degree of malabsorption. It appears that giardiasis produces a more severe degree of villous damage in patients with hypogammaglobulinemia. Giardiasis does not appear to be an important pathogen in patients with AIDS, although the infection has certainly been found in this group and in homosexual men.

In a study of 567 patients with giardiasis, a retrospective analysis of cases reported between 1988 and 1994 was performed. On histologic slides, trophozoites were found in duodenal (82.5%) and jejunal (2.1%) mucosa and also in gastric antral (8.7%) and ileal (12.1%) mucosa but rarely in the colon (0.4%). An entirely normal light-microscopic appearance of the duodenal mucosa was found in 462 patients (93.6%). Mild villous shortening and mild inflammation of the lamina propria were observed in the duodenal mucosa in 3.7% of the patients. Since the histology of the small-bowel mucosa is inconspicuous in the majority of patients with giardiasis, careful review of biopsy specimens must be performed (41).

Molecular Testing. In January 2013, the FDA approved the first test that can simultaneously detect 11 common viral, bacterial, and parasitic (*Cryptosporidium*, *Giardia*, *E. histolytica*) causes of infectious gastroenteritis from a single patient sample (xTAG Gastrointestinal Pathogen Panel; Luminex, Inc., Austin, TX) (42).



Algorithm 22.1 Giardiasis. Fecal immunoassay kits can be used on human stool specimens to confirm the presence of *Giardia* spp. Currently available reagents can be used with fresh, frozen, or preserved specimens (not those containing polyvinyl alcohol). If fluorescent testing is performed, the specimen should not be frozen (the actual organisms are disrupted during the freeze-thaw cycle); however, fresh or preserved specimens are acceptable. Check with each manufacturer for compatibility of the fixative with the proposed test; not all fixatives are compatible with all types of fecal immunoassays.

KEY POINTS—LABORATORY DIAGNOSIS

Giardia lamblia (*G. duodenalis*, *G. intestinalis*)

1. Even if a series of three stool specimens is submitted and examined correctly using the ova and parasite examination, the organisms may not be recovered and identified, particularly in a light infection.
2. Motility on wet preparations may be difficult to see because the organisms may be caught up in mucus. The only hint that *Giardia* is present may be the slight flutter of the flagella under reduced light. Do not use the ethyl acetate step in the concentration procedure if the specimen contains a lot of mucus.
3. Any examination for parasites in stool specimens must include the use of a permanent stained smear (even on formed stool).
4. Duodenal drainage (centrifuge and examine sediment; do not use the ethyl acetate step) and/or the use of the Entero-Test capsule may be very helpful in organism recovery. However, this technique does not take the place of the ova and parasite examination.
5. It has been well documented that the use of a fecal immunoassay (enzyme immunoassay, fluorescent-antibody assay, or rapid cartridge) does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia*; two different stool samples are recommended unless the first specimen is positive.
6. When performing the enzyme immunoassay, make sure that the wells are washed thoroughly according to directions; in order to avoid false-positive results, do not skip any wash steps. Make sure that the stream of buffer goes directly into the wells. Also, make sure that you use a wash bottle with a small opening, so that you have to squeeze the bottle to get the fluid to squirt directly into the wells.
7. When the directions tell you to “slap” the tray down onto some paper towels to remove all the fluid, make sure you slap it several times. Don’t be too gentle; the cups will not fall out of the holder. If they become loose, just push them back into the holder.
8. It is recommended that the fluorescent-antibody assay procedures be performed using centrifuged stool (500 × *g* for 10 min); prepare thin (rather than thick) smears and *perform the rinse step gently*. Remember that not all clinical specimens will provide the 3+ to 4+ fluorescence that we often see in the positive control. Also, from time to time, you may see fluorescing bacteria and/or some yeast in certain patient specimens. This is not particularly common, but the shapes can be distinguished from *Giardia* cysts and/or *Cryptosporidium* oocysts. Occasionally you may see the outline of a fluorescing trophozoite (some antigenic sites are shared between cysts and trophozoites); however, the fluorescence is often no more than a 1+ or maybe 2+. This finding can be interpreted as a positive test for *Giardia*. Normally, one can see cysts, even if a few trophozoites are present.
9. When using the cartridge (lateral-flow) test, if the stool is too thick, addition of reagents will not thin it out enough. If the specimen poured into the well remains too thick, the fluid will not flow up the membrane. If your specimens arrive in fixative and there is no fluid at the top of the vial overlaying the stool, this means that the vial may have been overfilled with stool. These specimens will have to be diluted with the appropriate diluent before being tested. It is always important to see the control line indicated as positive all the way across the membrane, not just at the edges. *A positive test result may be much lighter than the control line; this is normal.* At the cutoff time to read the result, any color visible in the test area should be interpreted as a positive. Do not read/interpret the results after the time indicated in the directions; you may get a false-positive result.

Treatment

The incidence of giardiasis worldwide may be as high as 1 billion cases. If giardiasis is diagnosed, the patient should

TABLE 22.5 Representative commercially available kits for immunodetection of *Giardia lamblia*

Diagnostic kit	Manufacturer and/or distributor[a]	Type of test[b]	Comments
ProSpecT Microplate assay	Remel (Thermo Scientific)	EIA	Different EIA formats (contact company for additional information)
Rapid assay, combination with *Cryptosporidium*	Remel (Thermo Scientific)		
GiardEIA	Antibodies Inc.	EIA	
PARA-TECT *Giardia*	Medical Chemical	EIA	Both DFA and EIA formats available; DFA is combination reagent (*Giardia*/*Cryptosporidium*)
PARA-TECT combination with *Cryptosporidium* spp.	Medical Chemical	DFA	
MeriFluor, combination with *Cryptosporidium* spp.	Meridian Bioscience	DFA	DFA is combination reagent (*Giardia*/*Cryptosporidium*)
Giardia Cel	Cellabs	DFA	
Giardia CELISA	Cellabs	EIA	
Crypto/Giardia Cel	Cellabs	DFA	DFA is combination reagent (*Giardia*/*Cryptosporidium*)
Giardia II	TechLab (Alere)	EIA	
Giardia/*Cryptosporidium* Chek	TechLab (Alere)	EIA	Combination reagent (*Giardia*/*Cryptosporidium*)
Giardia/*Cryptosporidium* Quik Chek	TechLab (Alere)	Rapid, cartridge (lateral flow)	Combination reagent (*Giardia*/*Cryptosporidium*)
Giardia Test	Wampole	EIA	
Triage Parasite Panel, combination test with *Cryptosporidium* and *Entamoeba histolytica*/*E. dispar* group	Biosite Diagnostics, Inc. (Alere)	Rapid, cartridge (lateral flow)	Requires fresh or frozen stool; combination test with *Cryptosporidium* and *Entamoeba histolytica*/*E. dispar* group
ColorPAC *Giardia*/*Cryptosporidium*	Becton Dickinson	Rapid, cartridge (lateral flow)	Can be used with fresh, frozen, or formalin-preserved stool; combination test with *Cryptosporidium* (some other fixatives also acceptable—check with company)
ImmunoCard STAT! *Cryptosporidium*/*Giardia*	Meridian Bioscience	Rapid, cartridge (lateral flow)	Can be used with fresh, frozen, or formalin-preserved stool; combination test with *Cryptosporidium* (some other fixatives also acceptable—check with company)
XPECT *Giardia*	Remel (Thermo Scientific)	Rapid, cartridge (lateral flow)	Can be used with fresh, frozen, or formalin-preserved stool (some other fixatives also acceptable—check with company)
XPECT *Giardia*/*Cryptosporidium*	Remel (Thermo Scientific)	Rapid, cartridge (lateral flow)	Can be used with fresh, frozen, or formalin-preserved stool; combination test with *Cryptosporidium* (some other fixatives also acceptable—check with company)

[a] Antibodies Incorporated, P.O. Box 1560, Davis, CA 95617-1560; Cellabs, P.O. Box 421, Brookvale, NSW 2100, Australia; Becton Dickinson Diagnostic Systems, 7 Loveton Circle, Sparks, MD 21152; Biosite Diagnostics, 11030 Roselle St., San Diego, CA 92121; Medical Chemical Corporation, 19430 Van Ness Ave., Torrance, CA 90501; Meridian Bioscience, 3471 River Hills Drive, Cincinnati, OH 45244; Remel, 12076 Santa Fe Drive, Lenexa, KS 66215; TechLab, 2001 Kraft Drive, Blacksburg, VA 24060-6358; Wampole Laboratories, 2 Research Way, Princeton, NJ 08540.

[b] EIA, enzyme immunoassay; DFA, direct fluorescent antibody; Rapid, cartridge, immunochromatographic lateral flow.

be treated (Table 22.6); more complete dosage information can be found in chapter 36. Current recommended treatments include the nitroheterocyclic drugs tinidazole, metronidazole, and furazolidone, the substituted acridine quinacrine, and the benzimidazole albendazole. Paromomycin is also used, as is nitazoxanide. Treatment failures have been reported with all the common antigiardial agents, and resistance to these drugs has been demonstrated in the laboratory. Clinical resistance has been reported, and includes situations in which patients have failed to respond to both metronidazole and albendazole treatments (43, 44). However, albendazole may be of similar effectiveness to metronidazole, may have fewer side effects, and has the advantage of a simplified regimen. Metronidazole is not recommended for pregnant women; although not well absorbed and not highly effective, paromomycin may be used to treat giardiasis in pregnancy (43). Tinidazole has also been used and has proven more effective than metronidazole as a single dose (43, 45). Less effective and/or less well-tolerated drugs for the treatment of giardiasis include albendazole, quinacrine, and furazolidone; the use of these agents should be reserved for giardi-

TABLE 22.6 Recommendations for therapy for giardiasis[a]

Clinical picture	Drug and duration of treatment	Side effects
Symptomatic infection, U.S.		
Adult and pediatric	Metronidazole, 5–7 days	Headache, vertigo, nausea, metallic taste, urticaria; disulfiram-like reaction with alcohol ingestion; rare side effects include pancreatitis, central nervous system toxicity, reversible neutropenia, peripheral neuropathy, T-wave flattening with prolonged use; mutagenic, carcinogenic?
Adult and pediatric	Nitazoxanide, single dose	Generally well tolerated
Alternatives	Furazolidone, 7–10 days	Nausea, vomiting, diarrhea; brown discoloration of urine; disulfiram-like reaction with alcohol ingestion; reacts unfavorably with monoamine oxidase inhibitors; mild hemolysis in glucose 6-phosphate dehydrogenase deficiency; carcinogenic?
	or quinacrine, 5–7 days	Nausea and vomiting, dizziness, headache; yellow/orange discoloration of skin, mucous membranes; rare side effects include toxic psychosis
	or albendazole, 5–7 days or single dose	Anorexia, constipation; rare side effects include reversible neutropenia and elevated liver function tests; teratogenic?
Symptomatic infection, non-U.S.		
Adult and pediatric	Tinidazole (single dose)	As for metronidazole
	or ornidazole (single dose)	As for metronidazole
Pregnancy		
First trimester	Paromomycin, 5–10 days	Ototoxicity and nephrotoxicity with systemic administration
Second and third trimesters	Paromomycin, 5–10 days, or metronidazole, 5–7 days	
Resistant infection or relapse	Drug of different class or combination nitroimidazole plus quinacrine, 2 wk or more	See above
Resistant infection (metronidazole)	Auranofin	Awaiting clinical trials to validate clinical efficacy in animals.

[a]Adapted from references 1, 15, 42, and 46.

asis refractory to treatment with the first-line agents (46). Auranofin, used for many years in treating rheumatoid arthritis, has been shown to be active against divergent *Giardia* strains with different forms of resistance against metronidazole, suggesting that auranofin could be a treatment alternative if patients fail standard metronidazole therapy (47). The ability of auranofin to override metronidazole resistance suggests that this drug has a mechanism of action different from that of metronidazole.

A simple colorimetric method has been determined for determining *in vitro* activity against *Giardia*. The microtiter plate assay is based on the nucleoside hydrolase activity released from the organism by lysis. Action of the nucleoside hydrolase on the substrate analog, 4-nitrophenyl β-D-ribofuranoside, gives rise to a colored product which can be determined by the change in absorbance. Values obtained using this method for metronidazole, tinidazole, and furazolidone were consistent with published values. This simple method, which does not involve radioisotopes or complex instrumentation, provided a convenient method for screening potential antigiardial agents (48).

Wheat germ (WG) agglutinin inhibits excystation and trophozoite growth *in vitro*, as well as reducing cyst passage in mice. In a double-masked, placebo-controlled study of dietary supplementation with WG, there appeared to be no clinically important differences between the two groups. However, symptoms appear to have resolved more rapidly in those taking WG in addition to metronidazole, and the WG was well tolerated in both the symptomatic and asymptomatic groups. Thus, in combination with antiprotozoal drugs, WG may be able to modify symptomatic giardiasis (49).

In the absence of a parasitologic diagnosis, the treatment of suspected giardiasis is a common question with no clear-cut answer. The approach depends on the alternatives and the degree of suspicion of giardiasis, both of which vary among patients and physicians. However, it is not recommended that treatment be given without a good parasitologic workup, particularly since relief of symptoms does not allow a retrospective diagnosis of giardiasis; the most commonly used drug, metronidazole, targets other organisms besides *Giardia*.

Another question involves treatment of asymptomatic patients. Generally, it is recommended that all cases of proven giardiasis be treated because the infection may cause subclinical malabsorption, symptoms are often periodic and may appear later, and a carrier is a potential source of infection for others. Certainly, in areas of the world where infection rates, as well as the prospect of reinfection, are extremely high, the benefit-per-cost ratio would also have to be examined.

Epidemiology and Prevention

Transmission is by ingestion of viable cysts. Although contaminated food or drink may be the source, intimate contact with an infected individual may also provide the infection mechanism. This organism tends to be found more frequently in children or in groups that live in close quarters. Often, there are outbreaks due to poor sanitation facilities or breakdowns as evidenced by infections of travelers and campers. There is also an increase in the prevalence of giardiasis in the male homosexual population, probably because of anal and/or oral sexual practices (43).

Although seasonal patterns have been identified for some infectious diseases, limited information is available for giardiasis. Some data suggest an association with the cooler, wetter months of the year; this is not surprising if one considers the issue of environmental conditions advantageous to cyst survival.

It has been documented that certain occupations may place an individual at risk for infection; such at-risk individuals include sewage and irrigation workers, who may become exposed to infective cysts. In situations where young children are grouped together, such as nursery schools, there may be an increased incidence of exposure and subsequent infection of both children and staff.

Most experts agree that the single most effective practice that prevents the spread of infection in the child care setting is thorough hand washing by the children, staff, and visitors. Specific guidelines can be found in Table 22.7. Rubbing the hands together under running water is the most important part of washing away infectious organisms. Premoistened towelettes or wipes and waterless hand cleaners should not be used as a substitute for washing hands with soap and running water. These guidelines are not limited to giardiasis but include all potentially infectious organisms.

TABLE 22.7 Guidelines for hand washing

- Children
  - When they arrive at the child care setting
  - Immediately before and after eating
  - After using the toilet or having their diapers changed
  - Before using water tables
  - After playing on the playground
  - After handling pets, pet cages, or other pet objects such as toys
  - Whenever hands are visibly dirty
  - Before going home
- Staff, visitors
  - When they arrive at the child care setting
  - Immediately before handling food, preparing bottles, or feeding children
  - After using the toilet, assisting a child in using the toilet, or changing diapers
  - After contact with a child's body fluids, including wet or soiled diapers, runny noses, spit, and vomit
  - After handling pets, pet cages, or other pet objects such as toys
  - Whenever hands are visibly dirty or after cleaning up a child, the room, bathroom items, or toys
  - After removing gloves for any purpose (if gloves are worn, hands should be washed immediately after the gloves are removed, even if the hands appear to be clean; use of gloves alone will not prevent contamination of hands or spread of organisms and should not be considered a substitute for hand washing)
  - Before giving or applying medication or ointment to a child or self
  - Before going home

The possible association between gastric acidity and giardiasis has been debated for some time. Evidence indicates that decreased gastric acid production may predispose people to *Giardia* infection. It is thought that normal gastric acidity acts as a barrier to the establishment of an infection; patients who have had a gastrectomy are prone to *Giardia* infection. Although achlorhydria is associated with blood group A and some evidence suggests that members of this group are more susceptible to giardiasis, subsequent evidence has not confirmed this information. Since reduction in gastric acid also occurs as a result of malnutrition, these factors may be linked and, as a group, increase the susceptibility to infection with this organism. This link between malnutrition and giardiasis may also be explained by the impairment of the host immune system.

The issue of whether breast milk modifies *Giardia* infection has also been discussed in terms of different interpretations of the results. A lower incidence of giardiasis in children younger than 6 months may be related to an association with breast-feeding and some protection against infection via secretory IgA; however, the lower incidence may also be related to decreased exposure to *Giardia* organisms in breast-fed infants.

Giardiasis is one of the more common causes of traveler's diarrhea and has been recorded in all parts of the world. It has also been speculated that visitors in areas where *Giardia* infections are endemic are more likely to present with symptoms than are individuals who live in the area; this difference is most probably due to the development of immunity of residents from prior and possibly continued exposure to the organism.

There have been a number of outbreaks attributed to either resort or municipal water supplies in Oregon, Colorado, Utah, Washington, New Hampshire, and New York (50–53). High rates of infection were also reported for hikers and campers who drank stream water. Because some of these areas were remote from human habitation, infected wild animals, especially beaver, are suspected (51).

During the past few years, this infection has received much publicity. With increased travel, there has been a definite increase in symptomatic giardiasis within the United States (53). Various surveys show infection rates of 2 to 15% in various parts of the world.

Because of the potential for wild-animal and possibly domestic-animal reservoir hosts, measures in addition to personal hygiene and improved sanitary measures have to be considered. Iodine has been recommended as an effective disinfectant for drinking water, but it must be used as directed. Filtration systems have also been recommended, although they have certain drawbacks such as clogging.

Water Testing. Over the past few years, concern over safe drinking water has increased, and these issues have led to the development of a more complex industry related to water treatment and testing. Untreated surface water is more likely than groundwater to contain organisms, because of the possibility of direct contamination with animal feces, treated and untreated human sewage, or fecal runoff from adjacent land after heavy rain or snow melt. Wells that have been poorly located, constructed, or damaged have also been implicated in outbreaks. Specific points to consider when testing well water are listed in Table 22.8; because specific testing for *Giardia* is difficult and expensive, the well water can also be tested for fecal coliforms as an indicator of fecal contamination. In some areas of the world, the percent positivity for water supplies can be very high for both *Cryptosporidium* and *Giardia* in both raw water and treated water samples.

The conventional filtration process used by most surface water treatment plants usually includes several steps: coagulation-flocculation, sedimentation, and filtration. During the first step, a chemical coagulant is added to the water to cause small suspended particles to stick together to form larger particles (flocculation), which either settle to the bottom (sedimentation) or are removed by filtration, primarily through fine sand. The coagulation-flocculation step is critical for successful removal of chlorine-resistant protozoa (1 to 20 µm in size), which could slip through the 50- to 70-µm spaces between grains of sand in a water treatment filter. Unfortunately, there are a number of variables that influence adequate filtration (amount of coagulant added, degree of mixing of coagulant with water, and changes in raw water pH, temperature, or turbidity). Conventional water filtration should trap nearly all protozoan parasites, including *Giardia* and *Cryptosporidium*, if appropriate procedures are followed (54).

TABLE 22.8 Questions for consideration when testing well water

1. Are other members of your family or users of your well water ill?
   (If yes, the well water may be a source of infection.)
2. Is your well located at the bottom of a hill, or is it considered shallow?
   (If either of these is true, runoff from rainwater or floodwater may be draining into the well, causing contamination.)
3. Is your well in a rural area where animals graze?
   (Well water can be contaminated with animal waste seepage contaminating the groundwater. This can occur if the well has cracked casings, is poorly constructed, or is too shallow.)

Environmental sampling methods were originally developed to assist in the investigation of suspected waterborne outbreaks of infection. The sample volumes generally recommended are 100 liters for source water and 1,000 liters or more for finished water. The usual procedure involves filtration through 1-µm polypropylene filter cartridges or fiberglass-resin cartridges. The filters are then eluted in a laboratory with detergent solutions, and recovered particulates are concentrated by centrifugation. A flotation purification step using density gradients is then used to separate the organisms from inorganic materials and debris. Currently, the most commonly used method for examining purified material for protozoa is an antibody-based immunofluorescence assay. This approach has several limitations, including the lack of information on the infectivity or viability of cysts or oocysts; the lack of indication of the host species or origin of the organisms; the chance of false identification of algal or other protozoal species; poor recovery efficiency; the labor-intensive procedures; and the need for skilled microscopists. It also appears that different organism numbers from the same water source can be attributed to method artifact rather than true differences in counts. Another method for the determination of *Giardia* cyst viability in environmental and fecal samples involves fluorogenic dye staining and differential interference contrast microscopy (55). This method has proved significantly better than the routine counting methods being used.

Newer methods that have been tried include a portable differential continuous-flow centrifuge for concentration of *Cryptosporidium* oocysts and *Giardia* cysts from large volumes of water (56). The effect of electroporation (very short pulses of high-voltage electricity) alone and in combination with various chemicals has been examined. While electroporation had a minor effect on oocyst and cyst survival, the combination of electrical and chemical treatment produced superior inactivation, particularly when chlorine, hydrogen peroxide, or potassium permanganate was used. Additional kinetic and optimum treatment combinations continue to be developed. The use of immunomagnetic beads to separate *Giardia* cysts from complex matrixes of environmental surface waters followed by DNA release and PCR amplification of the target giardin gene has improved the reliability of detection of *Giardia* with increased sensitivity (57). Water monitoring has undergone tremendous transition, with new molecular tools beginning to offer rapid, high-throughput, sensitive, and specific detection of a number of pathogens. High-density microarrays, quantitative real-time PCR (qPCR), and pyrosequencing are emerging rapidly as tools of pathogen detection and discovery (58).

Studies of the epidemic and endemic seroprevalence of antibodies to *Cryptosporidium* and *Giardia* in residents of three communities with different drinking water supplies were undertaken to review possible differences or similarities (59). The findings were consistent with a lower risk of exposure from drinking water obtained from deep well sources and increased seropositivity associated with an outbreak situation. Although intriguing associations were found among seroprevalence, outbreak-related serologic data, and patterns of parasite contamination of drinking water, further studies are required to validate this approach to risk assessment of waterborne parasitic infections at the community level.

Phagocytosis of *Giardia* cysts by hemocytes of the Asian freshwater clam, *Corbicula fluminea*, has been documented, and the mean number of cysts ingested per hemocyte increased significantly over time. These findings may result in this biological system being used to serve as an indication of contamination of wastewaters and agricultural drainage with *Giardia* cysts (60). Also, it is well known that rotifers thrive in environments such as wastewater and domestic sewage; they have been found to ingest *Giardia* cysts in an artificial setting. If this finding is consistent with results from natural environmental studies, perhaps the information will further explain the environmental dispersion of the cysts and suggest how this dispersion could be curtailed (61). Another study of blue mussels in Canada revealed *Giardia* (18%) and *Cryptosporidium* (73%) were found in the samples tested (62).

Characterization of *Giardia* Isolates. Isoenzyme studies, designed primarily to assist in organism identification and classification, have also provided additional information regarding pathogenicity, implication in waterborne outbreaks, and human disease. In one study in which isoenzyme patterns of 32 *Giardia* isolates obtained from both humans and animals were examined, there was no obvious correlation between clinical symptoms and isoenzyme patterns. Isolates from asymptomatic individuals were found in the same zymodemes (isoenzyme groups) as were isolates from symptomatic hosts. This study also confirmed previous observations regarding genetic heterogeneity and demonstrated significant differences between isolates from within a single region and other widely separated geographic locations (2). Australian data on two *Giardia* demes indicated that both appeared to be pathogenic and were derived from children with similar chronic symptoms (13).

Various studies have published data suggesting that zoonotic transmission between humans and animals is, at times, more likely or less likely to occur (5, 51, 63, 64).

Dientamoeba fragilis

Dientamoeba fragilis was first seen in 1909 but was not described until 1918. In 1940, Dobell recognized the close morphologic similarities between *D. fragilis* and *Histomonas meleagridis*, the ameboflagellate parasite of turkeys. A key scientific advance was made in 1934 when Tyzzer reported that *H. meleagridis* is transmitted in the eggs of *Heterakis*, the cecal worm of chickens and turkeys, a fact that has relevance to the life cycle of *D. fragilis* (1, 65). On the basis of electron microscopy studies, *D. fragilis* has been reclassified as an ameboflagellate rather than an ameba and is closely related to *Histomonas* and *Trichomonas* spp. (66). It has a cosmopolitan distribution, and past surveys demonstrate incidence rates of 1.4 to 19%. Much higher incidence figures have been reported for mental institution inmates, missionaries, and Native Americans in Arizona. *D. fragilis* tends to be common in some pediatric populations, and incidence figures are higher for patients younger than 20 years in some studies. Currently, it is in the phylum Parabasala, class Trichomonadea, family *Trichomonadidae*, and possibly the subfamily *Trichrichomonidinae*. This classification will probably undergo change in the future.

Life Cycle and Morphology

The life cycle and mode of transmission of *D. fragilis* were always speculative; however, newer information has clarified these issues. Transmission via helminth eggs such as those of *Ascaris* and *Enterobius* spp. has been postulated (65, 66) (Fig. 22.5). The cyst stage has recently been confirmed, thus also confirming fecal-oral transmission of *D. fragilis* (67–70, 82) (Tables 22.3 and 22.4).

Trophozoite. The trophozoite is characterized as having one (20 to 40%) or two (60 to 80%) nuclei. The nuclear chromatin is usually fragmented into three to five granules, and there is normally no peripheral chromatin on the nuclear membrane (Fig. 22.6 and 22.7). In some organisms, the nuclear chromatin tends to mimic that of *Endolimax nana*, *Entamoeba hartmanni*, or even *Chilomastix mesnili*, particularly if the organisms are overstained. The cytoplasm is usually vacuolated and may contain ingested de-

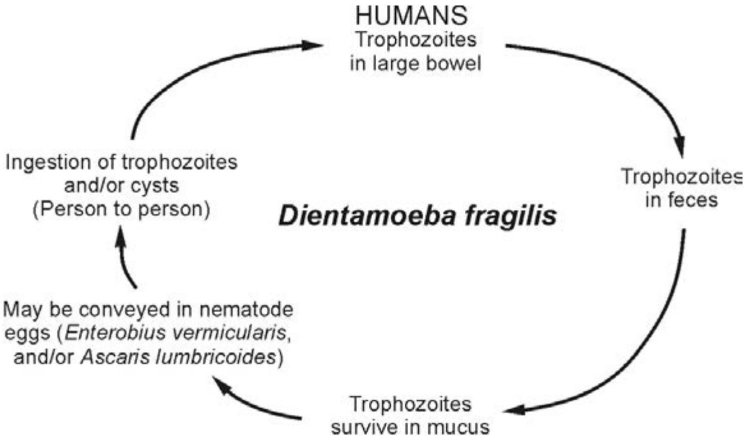
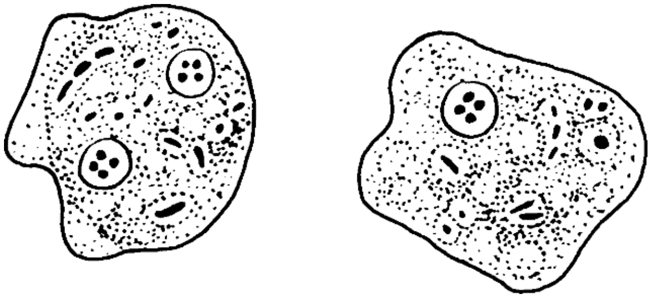


Figure 22.5 Life cycle of *Dientamoeba fragilis*. doi:10.1128/9781555819002.ch22.f5

bris as well as some large, uniform granules. The cytoplasm can also appear uniform and clean with few inclusions. There can also be considerable size and shape variation among organisms, even on a single smear.

Cyst. Electron microscopy reveals various organelles within the cyst, including an axostyle, flagellar axonemes, and a costa. External flagella are absent. Observation of flagella components only within the cyst and not in the trophozoite stage provides support for the suggestion that *D. fragilis* has adapted to life in the gut by losing the flagella and adopting an amebic appearance and style of locomotion in the gut (68, 69). Precysts and cysts are extremely difficult to identify and tend to be quite rare when compared with cyst numbers of other protozoa. The cysts have a distinct cyst wall (~5 µm in diameter) with a clear zone around the cyst. A space is present between the cyst wall and the organism enclosed within the cyst wall. The nuclear structure is morphologically identical to that found in *D. fragilis* trophozoites. All cysts seen contain two nuclei, with each nucleus containing a large central karyosome with a delicate nuclear membrane. No chromatin is visible on the nuclear membrane and the nucleus is often fragmented into distinct granules of chromatin. These "true" cysts are rarely encountered in clinical samples, which probably accounts for the limited number of descriptive reports. In contrast, the precystic forms of *D. fragilis* are more frequently seen with a prevalence of up to 5% in clinical samples. This precystic stage is characterized by a compact spherical shape with a reduction in size of up to 50% from "normal" trophozoites. These forms range in size from 4 to 5 µm. The cytoplasm is darkly staining, indicating a denser structure than what is found in normal trophozoites. The cytoplasm is homogeneous and rarely contains any inclusions (70) (Fig. 22.7).

Figure 22.6 Trophozoites of *Dientamoeba fragilis*. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch22.f6



Clinical Disease

D. fragilis has been associated with a wide range of symptoms (66, 68, 69). The development of a rodent model, the fulfilling of Koch's postulates, and the discovery of a cyst stage in the life cycle have dramatically clarified possible transmission routes, including fecal-oral transmission (67–70). Case reports of children infected with *D. fragilis* reveal a number of symptoms, including intermittent diarrhea, abdominal pain, nausea, anorexia, malaise, fatigue, poor weight gain, and unexplained eosinophilia. Eosinophilia has been reported in up to 50% of pediatric and 10% of adult patients. The most common symptoms in patients infected with this parasite appear to be intermittent diarrhea and fatigue. In some patients, both the organism and the symptoms persist or reappear until appropriate treatment is initiated. Chronic symptoms are common, with up to a third of patients exhibiting persistent diarrhea.

Eleven pediatric patients, seven of whom had peripheral eosinophilia and a history of recent travel, were diagnosed with *D. fragilis* infection and reported symptoms of anorexia, intermittent vomiting, abdominal pain, and diarrhea. Based on findings in these patients, including bovine protein allergy and eosinophilic colitis, *D. fragilis* should be included in the differential diagnosis of chronic diarrhea and eosinophilic colitis. The identification of this pathogen requires clinical awareness of epidemiologic risk factors and presenting complaints, as well as proper laboratory permanent staining procedures essential to proper identification. Long-term parasite carriage by rodents and prolonged shedding of cysts, together with elevated levels of calprotectin in the stool, confirms the capacity of this organism to cause disease. An increase in fecal calprotectin has been reported in patients suffering from intestinal disorders such as inflammatory bowel disease (71). This new information definitely indicates that dientamoebiasis should be considered in the differential diagnosis of gastrointestinal diseases, including inflammatory bowel disease.

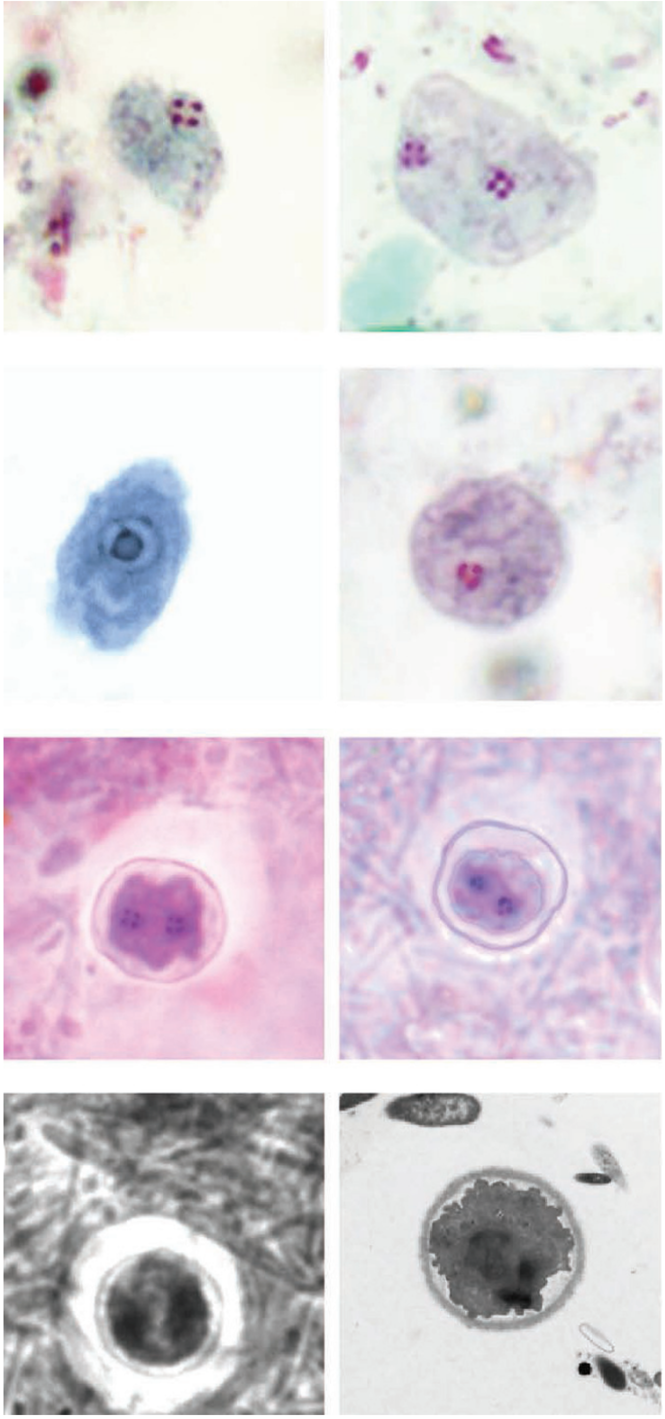


Figure 22.7 (Top) Trophozoites of *Dientamoeba fragilis*: (left) the trophozoite contains a single nucleus fragmented into four parts; (right) the trophozoite contains two nuclei, both of which are also fragmented. (Row 2, left) Trophozoite with a single nucleus that has not yet fragmented—can be confused with *Endolimax nana*; (right) trophozoite with a single nucleus that has begun to fragment. (Row 3, left and right) Cysts of *Dientamoeba fragilis*; note the space between the cyst wall and organism, also the two nuclei with fragmented chromatin. (Bottom, left) Cyst from rat feces; (right) transverse section across cyst showing the outer fibrillar cyst wall, peritrophic space, and the enclosed parasite. (Bottom row from reference 67 with permission).
doi:10.1128/9781555819002.ch22.f7

Diagnosis

Routine Diagnostic Procedures. Clinicians should include infection with *D. fragilis* in their differential diagnosis of patients presenting with abdominal pain, diarrhea, unexplained flatulence, nausea, vomiting, and/or eosinophilia. Diagnosis of *D. fragilis* infections depends on proper collection and processing techniques (a minimum of three fecal specimens) (27). Although the survival time for this parasite has been reported as 24 to 48 h, the survival time in terms of morphology is limited, and stool specimens must be examined immediately or preserved in a suitable fixative soon after defecation. It is particularly important that stained stained smears of stool material (trichrome, iron-hematoxylin) be examined with an oil immersion lens (100×). These organisms have been recovered in formed stool; therefore, a permanent stained smear must be prepared for every stool sample submitted for a parasite examination. Organisms seen in direct wet mounts may appear as refractile, round forms; the nuclear structure cannot be seen without examination of the permanent stained smear. With the recent confirmation of the cyst stage, one needs to take into account the more shrunken appearance of this form when compared with the trophozoite (Fig. 22.7).

Antigen Detection. Although fecal immunoassays for antigen detection are not yet available commercially, they have been developed using the immunofluorescence format (72). Studies using the enzyme immunoassay method are also under way. It is hoped that these assays may become available commercially, since preliminary results look very promising. The potential for detection of DNA from feces is also being investigated; certainly the development of rapid, specific, and sensitive tests would be extremely helpful within the diagnostic laboratory setting.

Antibody Detection. Using an indirect immunofluorescence assay, Chan et al. (73) found that serum samples from patients with confirmed *D. fragilis* infections had positive titers of 80, and all 12 matched controls had positive titers ranging from 20 to 160. The specificity of this assay was reinforced by immunoblotting 20 representative serum samples against *D. fragilis*; in all 17 indirect immunofluorescence-positive serum samples, a 39-kDa protein band of *D. fragilis* was identified.

Molecular Testing. The performance of a multiplex real-time PCR for the detection of *Blastocystis*, *D. fragilis*, *G. lamblia*, *Cryptosporidium* spp., and *Entamoeba* spp. in fecal samples was evaluated in an observational prospective study. Based on symptoms and clinical findings, pediatric patients suspected of having gastrointestinal parasitic disease were tested. Out of 163 patients, 114 (70%) had a fecal PCR-positive test. *D. fragilis* was detected most often, followed by *Blastocystis* (74).

Another study used a multiplex tandem PCR (MT-PCR) assay for the detection and identification of four common pathogenic protozoan parasites, *Cryptosporidium* spp., *D. fragilis*, *E. histolytica*, and *Giardia intestinalis*, from human clinical samples. In this study, *Giardia* ranked first, with *D. fragilis* being second in terms of positives. The MT-PCR assay provided rapid, sensitive, and specific simultaneous detection and identification of the four organisms. This study also emphasized the lack of sensitivity demonstrated by microscopy, and thus, molecular methods such as MT-PCR must be considered the diagnostic methods of choice for enteric protozoan parasites (75).

Since 2007, Statens Serum Institut (Denmark) has used real-time PCR for *D. fragilis* as the routine diagnostic work-up for intestinal parasitosis, testing more than 22,000 samples from 2008 through 2011. They demonstrated a very high proportion (43%) of investigated patients positive for *D. fragilis*, ranging from 12 to 71% depending on the age group, with a bimodal age distribution peaking in children and adults of parental age, as well as a clear association between exposure to children and risk of *D. fragilis* infection (76).

Recently, the EasyScreen Enteric Parasite Detection Kit (Genetic Signatures, Sydney, Australia) was evaluated for the detection and identification of five common enteric parasites—*Blastocystis* spp., *Cryptosporidium* spp., *D. fragilis*, *Entamoeba* complex, and *G. intestinalis*—in human clinical samples. This enteric parasite detection kit exhibited 92 to 100% sensitivity and 100% specificity and detected all commonly found genotypes and subtypes of these clinically important human parasites. No cross-reactivity was detected in stool samples containing various other bacterial, viral, and/or protozoan species (77).

KEY POINTS—LABORATORY DIAGNOSIS

Dientamoeba fragilis

1. A minimum of three specimens (stool) should be submitted for the diagnosis of *Dientamoeba* infections.
2. Although a cyst stage has been confirmed, these organisms will still be difficult to see on a wet preparation. Consequently, it is mandatory that a permanent stained smear be included in the ova and parasite examination. Trophozoites with either one or two nuclei can be found in the same specimen; there may also be tremendous size variation among the organisms seen in a single smear.
3. The trophozoite forms have been recovered from formed stool, hence the need to perform the permanent stained smear on specimens other than liquid or soft stools.
4. Remember, the cyst form will appear shrunken with two cyst walls; often there will be a large clear area surrounding the cyst (permanent stain). This is evidence of shrinkage during dehydration and is commonly seen with other protozoa as well.
5. Organisms with a single nucleus can easily be confused with *Endolimax nana* or *Entamoeba hartmanni*, both of which are considered nonpathogens.

Treatment

Clinical improvement has been observed in adults receiving tetracycline; symptomatic relief has been observed in children receiving either diiodohydroxyquin, metronidazole, or tetracycline. Current recommendations include iodoquinol, paromomycin, tetracycline, metronidazole, or combination therapy (78). Since symptomatic relief has been observed to follow appropriate therapy, *D. fragilis* is probably pathogenic in infected individuals who are symptomatic. Although limited studies have been undertaken regarding the efficacy of various therapies, information continues to support the fact that the elimination of this organism from symptomatic patients leads to clinical improvement. Additional information can be found in chapter 36.

Epidemiology and Prevention

As reported for many of the intestinal protozoa, *D. fragilis* is worldwide in distribution. It is suspected that the true incidence of this infection is considerably higher than reported, particularly since many laboratories do not yet emphasize diagnostic methods such as the permanent stained smear that would confirm the diagnosis (27, 69). Infection with *D. fragilis* is particularly common in Canada. This parasite has been associated with diarrhea and abdominal pain in some patients. In looking at seroprevalence data for a large group of randomly selected patients aged from 6 months to 19 years, 172 (91%) of 189 gave positive results at titers of 1:10 or higher. The specificity of the indirect immunofluorescence assay was reinforced by immunoblotting of 20 representative serum samples against *D. fragilis*. Findings over a 5-year period indicate that *D. fragilis* was the most common protozoan, followed closely by *G. lamblia* and more distantly by *Cryptosporidium parvum*. Previous data reported 85.6% for *G. lamblia* and 86% for *C. parvum* (72).

Since fecal-oral transmission has now been documented, preventive measures would tend to be those related to other intestinal pathogenic protozoa. With transmission occurring from the ingestion of certain helminth eggs and/or cyst forms, the use of hygiene and sanitary measures to prevent contamination with fecal material would be appropriate. There is speculation that *D. fragilis* may be infrequently recovered and identified; low incidence or absence from survey studies may be due to poor laboratory techniques and a general lack of knowledge about the organism (27, 66, 69).

Pentatrichomonas hominis (*Trichomonas hominis*)

It is generally thought that *Pentatrichomonas hominis* was first observed by Davaine in 1854 and described by him with the species name *hominis*. Although other protozoologists doubt that he actually saw this organism, the species name has been accepted. The genus name, *Pentatrichomonas*, is based on the five anterior flagella and a granular parabasal body. Although most readers are familiar with the generic term *Trichomonas*, this name will be changed to *Pentatrichomonas* for this edition of the book.

P. hominis has been recovered from all parts of the world, in both warm and temperate climates. It is considered to be nonpathogenic, although it is often recovered from diarrheic stools (Tables 22.3 and 22.4). Statistics generated over the years include college students (0.16%); rural Georgia (0.3%); New Orleans clinic population in 1935 (1.2%); Peking, China, clinic patients (3.5%); and a Cali, Colombia, survey (1956 to 1961) (2.0 to 13.5%) (2).

Life Cycle and Morphology

There is no known cyst stage (Fig. 22.8). The trophozoites live in the cecal area of the large intestine and feed on bacteria. The organism is not considered to be invasive. The trophozoite measures 5 to 15 µm in length and 7 to 10 µm in width. It has a pyriform shape and has both an axostyle and undulating membrane, which help in the identification (Fig. 22.3). The undulating membrane extends the entire length of the body, in contrast to that seen in *Trichomonas vaginalis* (Fig. 22.9).

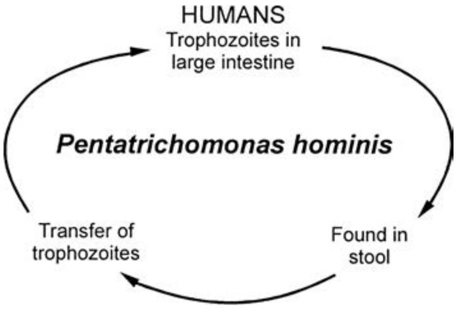
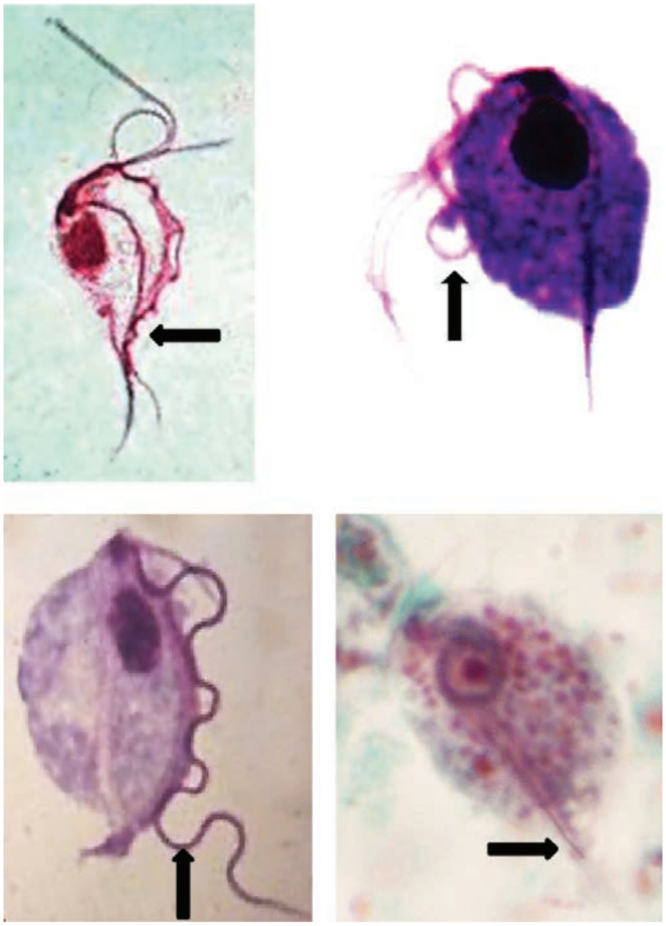


Figure 22.8 Life cycle of *Pentatrichomonas hominis*. doi:10.1128/9781555819002.ch22.f8

Figure 22.9 (Upper, left) *Pentatrichomonas hominis*, note undulating membrane extends the entire length of the body (arrow) (courtesy of http://umvf.univ-nantes.fr/parasitologie/enseignement/trichomonose/site/html/3.html); (right) *Trichomonas vaginalis*, note the undulating membrane extends about half the length of the body (arrow). (Lower, left) *P. hominis*, note undulating membrane extends the entire length of the body (arrow); (right) *P. hominis*, note the axostyle (supporting rod) protruding from the bottom (arrow). In this example, no undulating membrane or flagella are visible (Wheatley's trichrome stain). doi:10.1128/9781555819002.ch22.f9



Diagnosis

In freshly passed stool specimens, particularly in unformed stool, motility may be visible. In a wet preparation, one specifically looks for the flagellar movement from the undulating membrane and the presence of the axostyle. These small flagellates are difficult to stain and may even be missed on a permanent stained smear, especially if the stain is pale. In more formed stools, the trophozoites may round up (not encysted) and may not exhibit the characteristic motility seen in more active forms. When preserved fecal specimens are received in the laboratory, the permanent stained smear is the most sensitive method for finding and identifying *P. hominis*.

Misdiagnosis in Urinalysis

It is important to remember that *Trichomonas vaginalis* is often diagnosed in urine via the organism motility. Since the finding of *T. vaginalis* in the urine of children can imply the possibility of a sexual transmission, it is very important to keep in mind the possibility of fecal contamination within a child's urine due to collection issues. Thus *P. hominis* (nonpathogen in stool) could accidentally be misdiagnosed as *T. vaginalis* (pathogen/sexually transmitted flagellate) in urine. ANY DIAGNOSIS OF *T. VAGINALIS* IN A CHILD'S OR INFANT'S URINE SHOULD ALWAYS BE CONFIRMED; the misdiagnosis of *P. hominis* as *T. vaginalis* could be misinterpreted as a possible child abuse case.

Epidemiology and Prevention

Since there is no known cyst stage, transmission probably occurs in the trophic form. If ingested in a protecting substance such as milk, these organisms can apparently survive passage through the stomach and small intestine in patients with achlorhydria. *P. hominis* cannot be transplanted into the vagina, the natural habitat of *T. vaginalis*. Based on diagnostic test results, the incidence of this organism is relatively low, but it tends to be recovered more often than *Enteromonas hominis* or *Retortamonas intestinalis*. However, without the use of the permanent stained smear, the presence of *P. hominis* may be missed. The infection is diagnosed more often in warm climates and in children rather than adults. Because of the fecal-oral transmission route, preventive measures should emphasize improved hygienic and sanitary conditions. Although *P. hominis* is commonly found in dogs, puppies, and cats, there is no documented information related to possible transmission to humans.

Trichomonas tenax

Trichomonas tenax was first recovered from the mouth, specifically in tartar from the teeth. It is worldwide in distribution. There is no known cyst stage (Tables 22.3 and 22.4; Fig. 22.10). The trophozoite has a pyriform shape and is smaller and more slender than that of *P. hominis*. The undulating membrane extends almost the entire length of the body, similar to that seen in *P. hominis* (Fig. 22.11). There is also the typical axostyle and a single nucleus. *T. tenax* cannot survive passage through the stomach and cannot be established in the vagina. Although the exact mode of transmission is not known, it is assumed to be by direct contact or use of contaminated dishes and glasses. Prevalence rates vary from 0 to 25%, depending on opportunities for exposure and on oral hygiene.

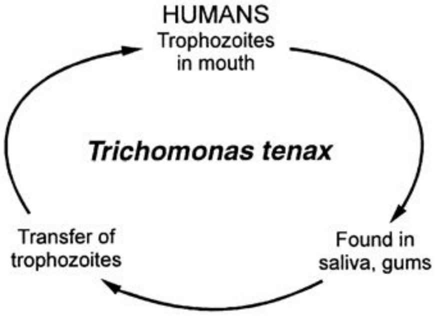


Figure 22.10 Life cycle of *Trichomonas tenax*. doi:10.1128/9781555819002.ch22.f10

Although *T. tenax* was considered a harmless commensal in the mouth, there are reports of periodontal disease, as well as respiratory infections and thoracic abscesses, particularly in patients with underlying cancers or other lung diseases. Studies have reported *T. tenax* as a cause of pleuropulmonary infections in humans. In a study from the Philippines using 18S rRNA amplification and sequencing, *T.*

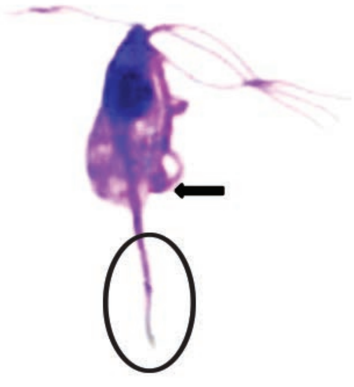


Figure 22.11 *Trichomonas tenax*, specimen from the mouth, Giemsa stain. Note the undulating membrane extends almost the entire length of the body (arrow). Also note the axostyle protruding from the posterior end (oval). In this example, the flagella are clearly visible. doi:10.1128/9781555819002.ch22.f11

tenax was found in 14 sputum specimens, thus confirming the presence of this organism in the respiratory tract of humans (79). Other cases were reported from western Europe. Diagnosis is based on recovery of the organisms from the teeth, gums, tonsillar crypts, or pulmonary specimens, and therapy may or may not be indicated. Better oral hygiene will rapidly eliminate the infection from the mouth.

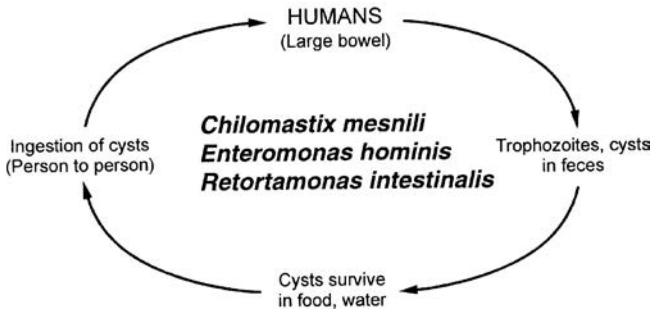
In another recent study, pleural infection was confirmed with *T. tenax* and a new *Tetratrichomonas* species was also detected in the oral cavity. The name *Tetratrichomonas empyemagena* as been proposed for this species (80).

Chilomastix mesnili

Chilomastix mesnili tends to have a cosmopolitan distribution, although it is found more frequently in warm climates. It has both trophozoite and cyst stages and is somewhat more easily identified than are some of the smaller flagellates such as *E. hominis* and *R. intestinalis* (Tables 22.3 and 22.4; Fig. 22.12). The trophozoite is described as being oval, measuring 6 to 24 µm in length and 4 to 8 µm in width. However, if the more elongate posterior end is not visible, the shape can be almost round. There is a single nucleus and a distinct oral groove or cytostome close to the nucleus (Fig. 22.3 and 22.13). Flagella are difficult to see unless there is obvious motility in a wet preparation. The morphology can be confirmed by using a permanent stained smear, particularly when the cytostome is visible. The cysts are pear or lemon shaped, and they range from 6 to 10 µm in length and 4 to 6 µm in width (Fig. 22.13). There is also a single nucleus in the cyst and the typical curved cytostomal fibril, which is called the shepherd's crook. Again, more definitive morphology can be seen on a permanent stain.

C. mesnili normally lives in the cecal region of the large intestine, where the organisms feed on bacteria and debris. It is considered to be a nonpathogen, and no treatment is recommended. However, as with all nonpathogenic protozoa, they should be reported to the physician; nonpathogens and pathogens are transmitted and acquired the same way. Thus, the result report indicates the patient has acquired something contaminated with fecal material (fecal/oral transmission). Particularly if the patient is symptomatic, additional fecal specimens should be examined for possible pathogens. Since transmission is through ingestion of infective cysts, prevention depends on improved personal hygiene and upgraded sanitary conditions.

Figure 22.12 Life cycle of *Chilomastix mesnili*, *Enteromonas hominis*, and *Retortamonas intestinalis*.
doi:10.1128/9781555819002.ch22.f12


HUMANS
(Large bowel)
Trophozoites, cysts in feces
Cysts survive in food, water
Ingestion of cysts (Person to person)
Chilomastix mesnili
Enteromonas hominis
Retortamonas intestinalis

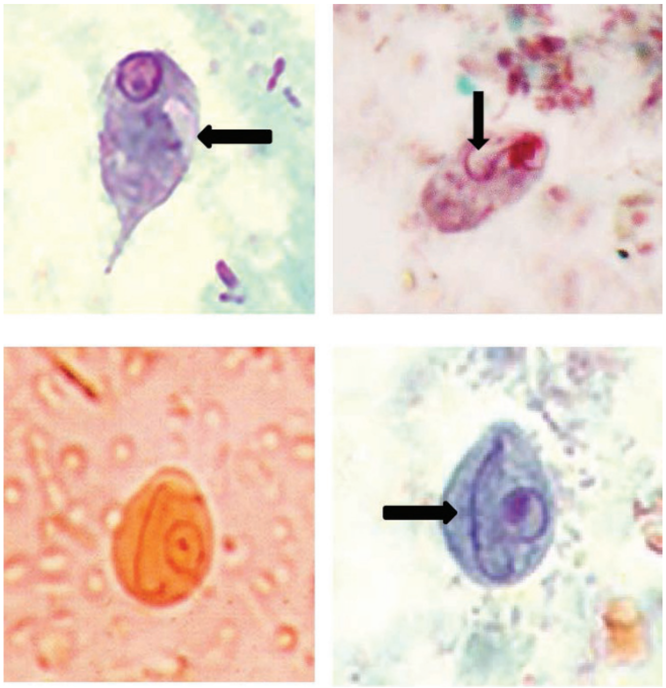


Figure 22.13 *Chilomastix mesnili*. (Upper, left and right) Trophozoites of *Chilomastix mesnili* (note the feeding groove; flagella are not visible) (arrows). (Bottom, left) *C. mesnili* cyst (iodine wet mount; nucleus and curved fibril are visible, as is the lemon shape); (right) *C. mesnili* cyst (note the "lemon or pear" shape and the curved fibril, the shepherd's crook) (arrow).
doi:10.1128/9781555819002.ch22.f13

Enteromonas hominis

The flagellate *E. hominis* is considered to be nonpathogenic and has been reported from both warm and temperate climates (0.2–0.8%) (Tables 22.3 and 22.4; Fig. 22.12). The trophozoites are somewhat pear shaped, measuring approximately 4 to 10 µm by 3 to 6 µm (Fig. 22.3). There is no cytostome, and the flagella are rarely visible unless motile organisms are seen. The cyst measures approximately 6 to 10 µm by 4 to 6 µm and tends to be oval. There are two nuclei, and the cyst may mimic a two-nucleated *E. nana* cyst. However, *E. nana* cysts containing two nuclei are quite rare in most clinical specimens. Also, *E. hominis* is not often reported, perhaps in part because of the small size of the organism and the difficulties in identification. Even on a permanent stained smear, this organism is often difficult to identify accurately. Infection is considered to be caused by ingestion of the infective cysts. No symptoms have been attributed to this infection, and therapy is not indicated.

Retortamonas intestinalis

Retortamonas intestinalis has been recovered from both warm and temperate areas of the world (Fig. 22.12). It has also been found in certain groups such as mental hospital patients. Like *E. hominis*, it is not commonly found in patient stools (Tables 22.3 and 22.4). *R. intestinalis* is also a small flagellate with trophozoites that are elongate pyriform or ovoidal (Fig. 22.3). They measure 4 to 9 µm in length by 3 to 4 µm in width. This flagellate does have a cytostome, which may be difficult to see, even in a permanent stained smear. The cysts are somewhat pear shaped, measuring 4 to 9 µm in length and 4 to 6 µm in width (Fig. 22.3). Both the trophozoite and the cyst have a single nucleus. This organism is rarely found in clinical specimens, possibly in part because of the difficulties in accurate identification of this and other smaller flagellates, even when the permanent stain technique is used. Infection is through ingestion of the cysts, and improved hygienic conditions would certainly prevent spread of the infection. *R. intestinalis* is considered a nonpathogen; thus, no therapy is indicated.

Balantidium coli

Balantidium coli was first isolated by Malmsten in 1857 from the dysenteric stools of two patients and was soon also observed by Leuckart in 1861 and Stein in 1862, who transferred the species to the genus *Balantidium* (prior genera were *Paramoecium*, *Leukophyra*, and *Holophyra*) (1).

B. coli is widely distributed in hogs, particularly in warm and temperate climates, and in monkeys in the tropics. Human infection is found in warmer climates, sporadically in cooler areas, and in institutionalized groups with low levels of personal hygiene. It is rarely recovered in clinical specimens within the United States.

Morphologically similar organisms have been found in a number of mammals, including rats, chimpanzees, orangutans, and, infrequently, dogs or cats. The species found in pigs, *Balantidium suis*, is probably identical to *B. coli* from humans. Other species have been isolated from guinea pigs (*Balantidium caviae*), cockroaches (*Balantidium blattarum*), fish, birds, and amphibia. In all, about 50 species have been described.

Life Cycle and Morphology

B. coli is the only pathogenic ciliate and is the largest of the protozoa that parasitize humans (Tables 22.9 and 22.10; Fig. 22.14). Both the trophozoite and cyst forms are found (81).

Trophozoite. The trophozoite is quite large, oval, and covered with short cilia; it measures approximately 50 to 100 µm in length and 40 to 70 µm in width (Fig. 22.15 and 22.16). The organism can easily be seen in a wet preparation on lower power (10× objective). The anterior end is somewhat pointed and has a cytostome present; in contrast, the posterior end is broadly rounded. The cytoplasm contains many vacuoles with ingested bacteria and debris. There are two nuclei within the trophozoite, a very large bean-shaped macronucleus and a smaller round micronucleus (more difficult to see). The organisms normally live in the large intestine. The motile trophozoite exhibits a rotary-type motion by means of its somatic cilia that may facilitate movement through the contents of the colon.

Cyst. The cyst is formed as the trophozoite moves down the intestine. The process of encystment begins in the colon and rectum of the host, and cysts are generally found in

TABLE 22.9 Intestinal protozoa: ciliate

Balantidium coli	Shape and size	Motility	No. of nuclei	Other features
Trophozoite	Ovoid with tapering anterior end; 50–100 µm in length; 40–70 µm in width (usual range, 40–50 µm)	Ciliates: rotary, boring; may be rapid	1 large kidney-shaped macronucleus; 1 small, round micronucleus, which is difficult to see even in the stained smear; macronucleus may be visible in unstained preparation	Body covered with cilia, which tend to be longer near cytostome; cytoplasm may be vacuolated
Cyst	Spherical or oval; 50–70 µm (usual range, 50–55 µm)		1 large macronucleus visible in unstained preparation; micronucleus difficult to see	Macronucleus and contractile vacuole are visible in young cysts; in older cysts, internal structure appears granular; cilia difficult to see within the cyst wall

TABLE 22.10 Intestinal flagellates and ciliates: recommended diagnostic procedures

Organism	Specimen	Diagnostic procedure
Giardia lamblia (*G. duodenalis*, *G. intestinalis*)	Stool	Complete ova and parasite exam, fluorescent antibody, enzyme and immunochromatographic immunoassays; approved molecular tests/instruments
	Duodenal contents	Wet preparation and permanent stains
	Entero-Test	Wet preparation and permanent stains
	Aspirate	Wet preparation and permanent stains
	Biopsy	Routine histology
Dientamoeba fragilis	Stool	Complete ova and parasite exam
Enteromonas hominis	Stool	Complete ova and parasite exam
Retortamonas intestinalis	Stool	Complete ova and parasite exam
Chilomastix mesnili	Stool	Complete ova and parasite exam
Pentatrichomonas hominis (*Trichomonas hominis*)	Stool	Complete ova and parasite exam
Trichomonas tenax	Gingival scrapings	Wet preparation and permanent stains
	Pyorrheal material	Wet preparation and permanent stains
	Pulmonary specimens	Wet preparations and permanent stains
Balantidium coli	Stool	Complete ova and parasite exam
	Pulmonary specimens[a]	Wet preparations and permanent stains
	Biopsy	Routine histology

[a] It is very important to differentiate between ciliated epithelial cells and trophozoites of *B. coli*. Bronchoalveolar wash fluid often contains these ciliated human cells, which can easily be confused with ciliates in wet preparations.

formed feces. Nuclear division does not occur in the cyst; therefore, only two nuclei are present, the macronucleus and the micronucleus (Fig. 22.15 and 22.16). The cysts measure from 50 to 70 μm in diameter.

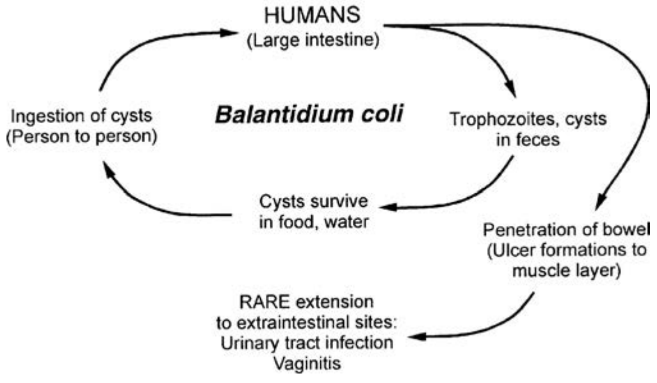
Clinical Disease

Some individuals with *B. coli* infections are totally asymptomatic, whereas others have symptoms of severe dysentery similar to those seen in patients with amebiasis. Although many patients are asymptomatic, malnutrition, alcoholism, or a compromised immune system can favor the parasite.

Symptoms usually include diarrhea or dysentery, tenesmus, nausea, vomiting, anorexia, and headache. Insomnia, muscular weakness, and weight loss have also been reported. The diarrhea may persist for weeks to months prior to the development of dysentery. There may be tremendous fluid loss, with a type of diarrhea similar to that seen in cholera or in some coccidial or microsporidial infections. Following ingestion, excystation releases trophozoites that invade the colonic mucosa, multiply, and form colonies. Within the tissue, *B. coli* propagates and may produce ulcers and abscesses that extend to the muscular layer. The symptoms of *B. coli*-induced colitis may be confused with amebic colitis. If extensive damage occurs in the wall of the colon, red blood cells may be seen within the trophozoites, as well as in the stool. *B. coli* produces no known toxins, but the ability to produce colonic ulcers is attributed to hyaluronidase, which is an enzyme that digests hyaluronic acid, a component of the "glue" holding mucosal epithelial cells together.

On contact with the mucosa, *B. coli* may penetrate the mucosa with cellular infiltration in the area of the developing ulcer (Fig. 22.17). Some of the abscess formations may extend to the muscular layer. The ulcers may

Figure 22.14 Life cycle of *Balantidium coli*.
doi:10.1128/9781555819002.ch22.f14



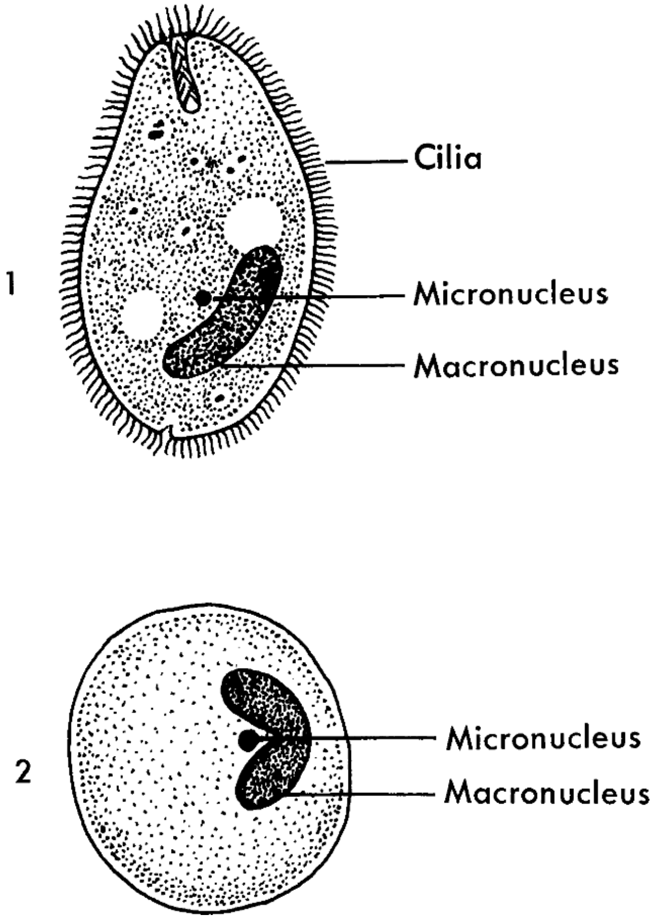


Figure 22.15 (1) Trophozoite of *Balantidium coli*; (2) cyst of *B. coli*. doi:10.1128/9781555819002.ch22.f15

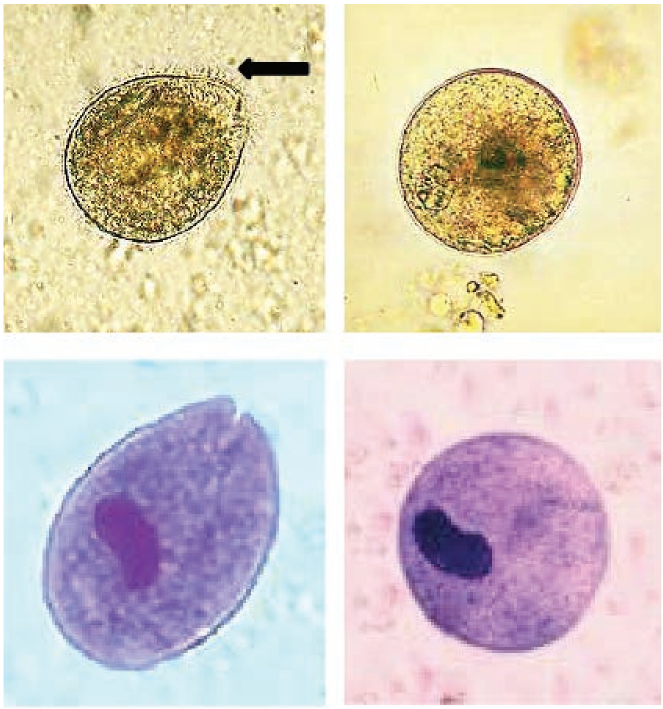


Figure 22.16 *Balantidium coli.* (Upper, left) Trophozoite in wet mount (note surrounding cilia shadows) (arrow); (right) cyst in wet mount (note the macronucleus is visible, but no cilia are visible within the cyst wall). (Lower, left) Trophozoite, stained, note the large macronucleus (in some permanent stained smears, the organism stains so dark, it will mimic a helminth egg); (right) cyst, stained (note very dark macronucleus, micronucleus is usually not visible within the trophozoite or cyst). doi:10.1128/9781555819002.ch22.f16

vary in shape, and the ulcer bed may be full of pus and necrotic debris. Although the number of cases is small, extraintestinal disease has been reported (peritonitis, urinary tract infection, inflammatory vaginitis, pulmonary hemorrhage and respiratory failure, and vertebral osteomyelitis and myelopathy).

Immunocompromised individuals have developed balantidiosis without any direct contact with pigs, perhaps with rats or contaminated produce as a possible source of infection. For the clinician, this infection should be included in the differential diagnosis for persistent diarrhea in travelers to or from Southeast Asia, the Western Pacific islands, rural South America, or communities where close contact with domestic swine occurs.

Death rarely occurs, but in developing countries with undernourished and overparasitized populations, this infection can make the difference between a healthy life and chronic debilitation. It has been suggested that there may be virulent and avirulent stains of *B. coli*; however, no solid evidence of this fact has been documented.

Diagnosis

Routine Stool Examination. Routine stool examinations, particularly wet-preparation examinations of fresh and concentrated material, will demonstrate the organisms. Organism recognition and identification on a permanent stained smear may be very difficult. These protozoa are so large that they tend to stain very darkly, thus obscuring any internal morphology. *B. coli* organisms may even be confused with helminth eggs because of their size, particularly when the cilia are not visible. The recovery of *B. coli* from specimens within the United States is rare. Nonetheless, laboratories are expected to be able to identify these organisms and are called upon to do so with proficiency-testing specimens.

Pulmonary Specimen Wet Preparations. Diagnosing lung infections with *B. coli* can be difficult due to possible confusion between ciliated epithelial cells and ciliate trophozoites. Bronchoalveolar wash fluid containing *Balantidium* may

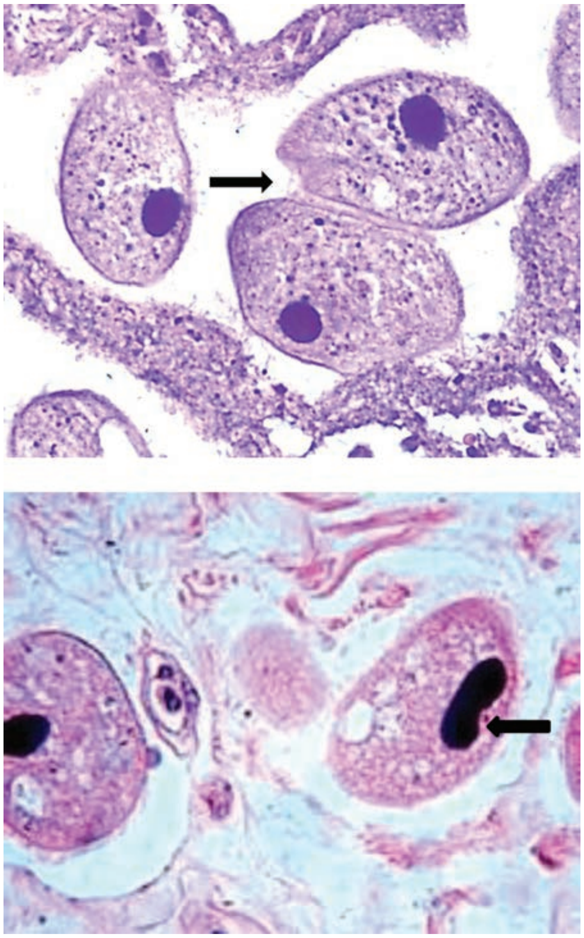


Figure 22.17 Trophozoites of *Balantidium coli*. (Upper) Tissue of pig large intestine. The cilia are visible surrounding the trophozoites; also note the oral groove (arrow). (Image courtesy of Dr. Forey, Veterinary Medicine, Washington State University/Pullman.) (Lower) Trophozoite in human colon (note large macronucleus; some cilia are visible on the lower trophozoite [arrow]). (Image courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch22.f17

also contain motile ciliated human cells from the trachea that can be mistaken for ciliates in wet mounts. A ciliated epithelial cell has few cilia on the surface compared to the uniformly ciliated surface of a *B. coli* trophozoite. Also, the cilia may be clumped in the case of columnar epithelial cells. Ciliated human cells generally measure <30 µm, while *B. coli* measures 150 to 200 µm, is elongated rather than ovoid, and is more likely to swim aimlessly in circles, unlike the pronounced spiraling movements of ciliates (81).

KEY POINTS—LABORATORY DIAGNOSIS

Balantidium coli

1. Since the organisms are so large, they can frequently be seen under low power (100×, 10× objective), particularly in a concentration sediment wet mount.
2. If fecal specimen wet mounts are examined using high dry power (400×, 40× high dry objective), the organisms may be confused with vegetable cells (both cells and parasites are larger than other intestinal protozoa). The cilia tend to be short and can be missed on microscopic examination.
3. Although this infection is very rare within the United States, all laboratories may receive positive specimens for proficiency testing; generally the specimens are designed to be examined as wet mounts only.
4. These organisms do not stain well (too large and thick) on the permanent stained smear (particularly if the smear is thick) and can be confused with fecal debris (including helminth eggs), hence the need to make the diagnosis by using wet smears (from direct mounts or concentrate sediment).
5. If pulmonary specimens are received, it is very important to differentiate between ciliated epithelial cells and true *B. coli* trophozoites.

Treatment

For treatment of *B. coli*, tetracycline is the drug of choice, although it is considered investigational for this infection. Iodoquinol or metronidazole may be used as alternatives. Additional information can be found in chapter 36.

Epidemiology and Prevention

Although *B. coli* has been found in many different simian hosts, domestic hogs probably serve as the most important reservoir host for human infection. In areas where pigs are the main domestic animal, the incidence of human infection can be quite high. Particularly susceptible to infection are persons working as pig farmers or in slaughterhouses (28% infection in New Guinea). Human infection is fairly rare in temperate areas, although once the infection is established, it can develop into an epidemic, particularly in areas with poor environmental sanitation and personal hygiene. This situation has been seen in mental hospitals in the United States. Preventive measures involve increased attention to personal hygiene and sanitation measures, since the mode of transmission is ingestion of infective cysts through contaminated food or water.

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23

Intestinal Protozoa (Coccidia), Microsporidia, and Algae

Coccidia
Cryptosporidium spp.
Cyclospora cayetanensis
Cystoisospora (*Isospora*) *belli*
Sarcocystis spp.
Microsporidia
Algae (*Prototheca*)

Coccidia

Although most readers understand the main categories related to parasitology classification, the coccidia and microsporidia can be somewhat confusing; a few comments as review may be helpful. The major phyla of protozoa include Sarcomastigophora (includes the amebae and flagellates), the Apicomplexa (malaria parasites, coccidia, and *Babesia*), and the Ciliophora (*Balantidium coli*). Several protozoan genera are included in the phylum Apicomplexa and are referred to as coccidia. All organisms included in the Apicomplexa are unicellular, with an apical complex typically composed of polar rings, rhoptries, micronemes, and usually a conoid; subpellicular microtubules and micropores are common. These structures can be seen in electron microscopy (EM) studies and are used to help classify the various coccidia. Genera that develop in the gastrointestinal tract of vertebrates throughout their entire life cycle include *Eimeria*, *Cystoisospora*, *Cyclospora*, and *Cryptosporidium*. Those that are capable of or require extraintestinal development are referred to as cyst-forming coccidia and include *Besnoitia*, *Caryospora*, *Frenkelia*, *Hammondia*, *Neospora*, *Sarcocystis*, and *Toxoplasma*. The coccidian genera that cause disease in humans include *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, *Sarcocystis*, and *Toxoplasma* (see chapter 25 for a discussion of *Toxoplasma*).

Cryptosporidium spp.

The first reported description of *Cryptosporidium parvum* was in 1907 in the gastric crypts of a laboratory mouse (Tyzzer). Subsequently, it has been found in chickens, turkeys, mice, rats, guinea pigs, horses, pigs, calves, sheep, rhesus monkeys, dogs, cats, kangaroos, lizards, snakes, and humans (Table 23.1) (1–4). Information also supports previous suggestions that cryptosporidiosis is a zoonosis, is not host specific, and is transmitted via the fecal-oral route (4). What was previously called *Cryptosporidium parvum* and was thought to be the primary *Cryptosporidium* species infecting humans is now classified as two separate species, *C. parvum* (mammals, including humans) and *Cryptosporidium hominis* (primarily humans) (3) (Table 23.1). Differentiation of these two species based on oocyst morphology is not possible using routine stains.

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TABLE 23.1 Current species names within the genus *Cryptosporidium*[a]

Species	Main host(s)	Less common host(s)
C. andersoni	Cattle, Bactrian camels	Sheep
C. baileyi	Chicken, turkeys	Cockatiels, quails, ostriches, ducks
C. bovis	Cattle	
C. canis	Dogs	Humans
C. fayeri	Kangaroos (red kangaroo)	
C. felis	Cats	Humans, cattle
C. fragile	Asian toad	
C. galli	Finches, chicken, capercaillies, grosbeaks	
C. hominis	Humans, monkeys	Dugongs, sheep
C. macropodum	Kangaroos (grey kangaroo)	
C. meleagridis	Turkeys, humans	Parrots
C. molnari	Fish	
C. muris	Rodents, Bactrian camels	Humans, rock hyrax, mountain goats
C. parvum	Cattle, sheep, goats, humans	Deer, mice, pigs
C. pestis	Cattle	
C. ryanae	Cattle	
C. saurophilum	Lizards	Snakes
C. scophthalmi	Fish	
C. serpentis	Snakes, lizards	
C. suis	Pigs	
C. waranii	Monitor lizards	
C. wrairi	Guinea pigs	

[a] Adapted from references 1–4.

Cryptosporidium infection is now well recognized as causing disease in humans, particularly in those who are in some way immunosuppressed or immunodeficient. *Cryptosporidium* has been implicated as one of the more important opportunistic agents seen in patients with AIDS (3–5). Also, reports of respiratory tract and biliary tree infections confirm that the developmental stages of this organism are not always confined to the gastrointestinal tract.

Unfortunately, at the time of this writing, no totally effective therapy for cryptosporidiosis has been identified, despite the testing of over 100 drugs. In the 25 years since the emergence of cryptosporidiosis as a significant disease, particularly in individuals with HIV/AIDS and other immunodeficient individuals, little progress has been made with the development of effective treatments. However, the sequencing of the genomes of *C. parvum* and *C. hominis* has led to the identification of new molecular targets for possible drug development. Currently, the literature contains over 4,500 published papers related to cryptosporidiosis, including information on nosocomial transmission, day care center outbreaks, and a number of waterborne outbreaks. This is remarkable, since fewer than 30 publications were available prior to 1980. In response to this rapidly expanding area of parasitology, there have been definite improvements in diagnostic procedures, particularly those involving the newer immunoassay detection kits (4, 6, 7).

The pathogenesis of cryptosporidial diarrhea, even in patients with AIDS, is not fully understood. However, patients with no intestinal symptoms who are passing formed stool containing few oocysts contrast sharply with those who are symptomatic with watery diarrhea and are passing large numbers of oocysts. These findings imply a relationship between intestinal dysfunction and injury and the number of organisms infecting the intestinal mucosa.

Age and immune status at the time of primary exposure to *Cryptosporidium* do not appear to be primary factors influencing the susceptibility of humans to infection. Symptomatic intestinal and respiratory cryptosporidiosis has been seen in both immunocompetent and immunodeficient patients of all ages. However, once the primary infection has been established, the immune status of the host plays a very important role in determining the length and severity of the illness. People who are immunocompetent usually develop a short-term, self-limited diarrhea lasting approximately 2 weeks.

In contrast, those who are immunocompromised initially develop the same type of illness; however, it becomes more severe with time and results in a prolonged, life-threatening, cholera-like illness. These severe infections have been seen in patients undergoing immunosuppressive chemotherapy with drugs that affect both T- and B-lymphocyte function, in patients with hypogammaglobulinemia, and in those with AIDS. From many observations of these types of patients, the difference in outcome can probably be explained by the development, in the immunocompetent host, of an immune response sufficient to eradicate the parasite from the infected individual. This explanation is also supported by the fact that individuals whose immunosuppressive therapy has been discontinued will rapidly clear the body of *Cryptosporidium* once their immune system function is restored. Studies also suggest that the overall immune response to cryptosporidiosis is probably an antibody-dependent, cell-mediated, cytotoxic effect of unknown mechanism. Patients with $CD4^+$ counts of less than 50 cells/mm^3 can develop persistent or intractable diarrhea, thus emphasizing the importance of the host immune response in controlling the disease.

Life Cycle and Morphology

Cryptosporidium differs from other coccidia infecting warm-blooded vertebrates in that its developmental stages do not occur deep within host cells but are confined to

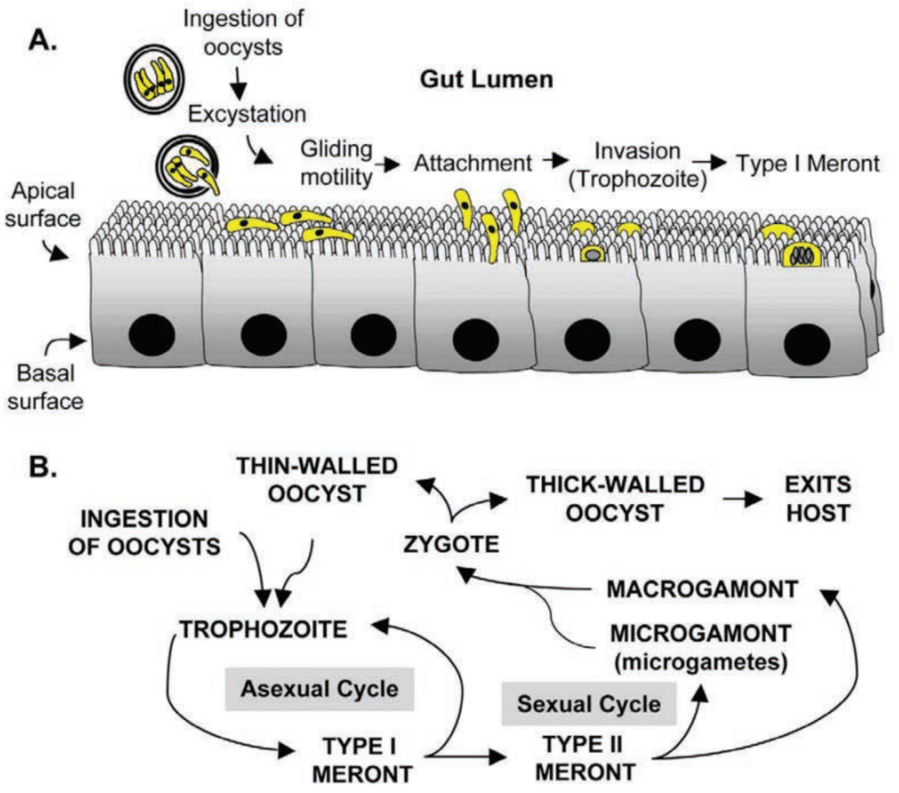


Figure 23.1 Life cycle of *Cryptosporidium* spp. illustrating various stages. (Illustration by Sharon Belkin.)
doi:10.1128/9781555819002.ch23.f1

an intracellular, extracytoplasmic location. Each stage is within a parasitophorous vacuole of host cell origin; however, the vacuole containing the organism is located at the microvillous surface of the host cell. Details of the life cycle can be seen in Fig. 23.1 and 23.2 (8).

The presence of a thin-walled, autoinfective oocyst may explain why a small inoculum can lead to an overwhelming infection in a susceptible host and why immunosuppressed patients may have persistent, life-threatening infections in the absence of documentation of repeated exposure to oocysts (Fig. 23.2). The stages found on the microvillous surface measure 1 μm, and the oocysts recovered in stool specimens measure 4 to 6 μm (Table 23.2; Fig. 23.3).

Each intracellular stage of *Cryptosporidium* resides within a parasitophorous vacuole within the microvillous region of the host cell. Oocysts undergo sporogony while they are in the host cells and are immediately infective when passed in the stool. This is in contrast to the oocysts of *Cystoisospora* (*Isospora*) *belli*, which do not sporulate until they are passed from the host and are exposed to oxygen and temperatures below 37°C. Approximately 20% of the oocysts of *Cryptosporidium* do not form the thick, two-layered, environmentally resistant oocyst wall. The four sporozoites within this autoinfective stage are surrounded by a single-unit membrane. After being released from a host cell, this membrane ruptures and the invasive sporozoites penetrate the microvillous region of other cells within the intestine and reinitiate the life cycle. As mentioned above, these thin-walled oocysts that can recycle are thought to be responsible for the development of severe, life-threatening disease in immunocompromised patients, even those who are no longer exposed to the environmentally resistant oocysts.

The prepatent period from the time of ingestion of as few as 10 infective oocysts to completion of the life cycle with excretion of newly developed oocysts in the human is approximately 4 to 22 days. The patent period during excretion of oocysts can range from 1 to 20 days in humans. Previous studies have suggested that persons infected with *Cryptosporidium* develop antibody responses to several antigens. Results now suggest that characteristic antibody responses develop following infection and that persons with preexisting antibodies may be less likely to develop illness, particularly when infected with low oocyst doses (4, 5).

Oocysts recovered in clinical specimens are difficult to see without special staining techniques, such as the modified acid-fast, Kinyoun, and Giemsa methods, or the fecal immunoassay methods. The four sporozoites may be seen within the oocyst wall in some of the organisms, although they are not always visible in freshly passed specimens (Fig. 23.4). The oocysts are immediately infective, regardless of whether or not the sporozoites are seen within every oocyst.

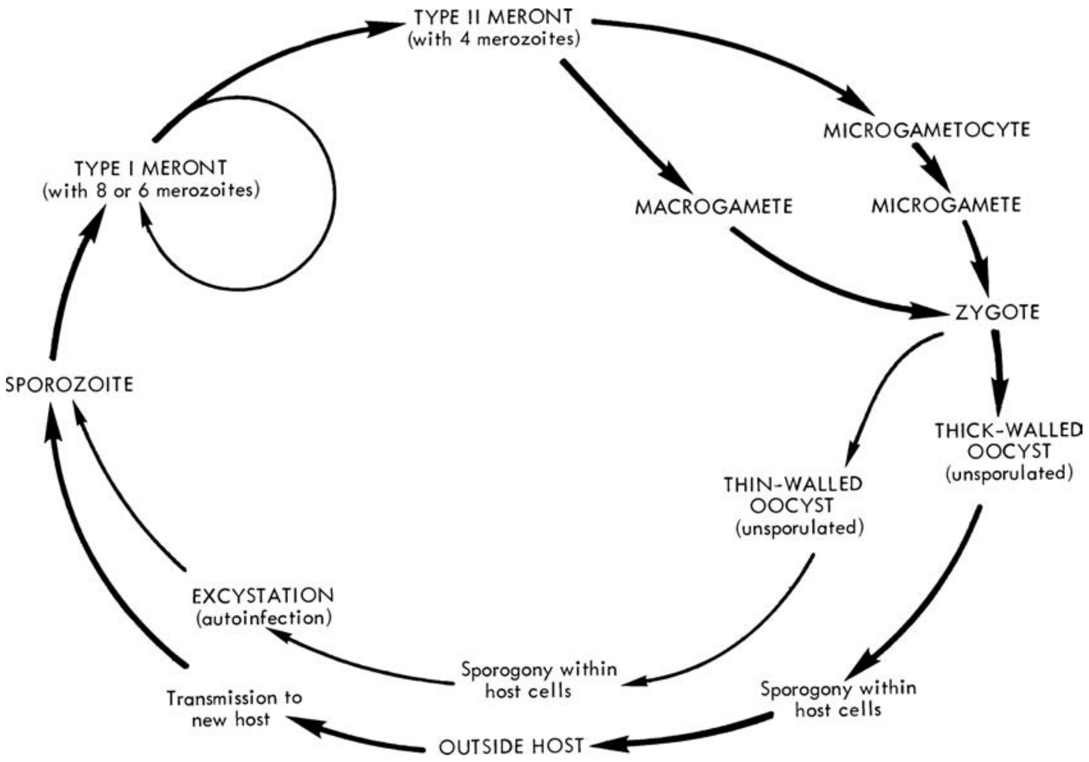

TYPE II MERONT
(with 4 merozoites)
MICROGAMETOCYTE
TYPE I MERONT
(with 8 or 6 merozoites)
MACROGAMETE
MICROGAMETE
ZYGOTE
SPOROZOITE
THICK-WALLED
OOCYST
(unsporulated)
THIN-WALLED
OOCYST
(unsporulated)
EXCYSTATION
(autoinfection)
Sporogony within
host cells
Sporogony within
host cells
Transmission to
new host
OUTSIDE HOST

Figure 23.2 Life cycle of *Cryptosporidium* spp. (Adapted from Current WL, Garcia LS. *Clin Microbiol Rev* **4**:325–358, 1991.) doi:10.1128/9781555819002.ch23.f2

Information Based on EM. The oocyst wall has distinct inner and outer layers and is unique in having a suture at one end; the suture dissolves during excystation, opening the wall, through which the sporozoites leave the oocyst. Excystation of sporozoites is enhanced by exposure to reducing conditions followed by exposure to pancreatic enzymes and/or bile salts. Data suggest that a novel parasite protein, CP47, may play an important role in attachment of sporozoites to the host cell; this protein exhibits significant binding affinity for the surfaces of both human and animal ileal cells. Both sporozoites and merozoites appear similar to those of other coccidia, with organelles such as the pellicle, rhoptries, micronemes, electron-dense granules, nucleus, ribosomes, subpellicular microtubules, and apical rings. Once they are inside the parasitophorous vacuole, changes in the apex of the host cell and the parasite result in an attachment or feeder organelle. The fertilized macrogamont, or zygote, develops into an oocyst with either a thick wall (80%, which are passed in the stool) or a thin wall (20%, which remain internal, causing internal autoinfection). The zygotes that develop into thin-walled oocysts do not contain the characteristic wall-forming bodies. The thick-walled oocysts are characterized by having a thin, moderately coarse outer layer and a finely granular inner layer. Between these two layers of the oocyst wall is an electron-lucent zone that consists of the two oocyst membranes sandwiched between the outer and inner layers of the oocyst wall. The outer layer of the wall is continuous and of a uniform thickness, while the inner layer contains a suture at one pole.

Clinical Disease

Immunocompetent Individuals. Clinical symptoms include nausea, low-grade fever, abdominal cramps, anorexia, and 5 to 10 watery, frothy bowel movements per day, which may be followed by constipation. Some patients may present with diarrhea as described above, and others may have relatively few symptoms, particularly later in the course of the infection. In patients with the typical watery diarrhea, the stool specimen contains very little fecal material but consists mainly of water and mucus flecks. Often the organisms are entrapped in the mucus, and diagnostic procedures are performed accordingly. Generally, a patient with a normal immune system has a self-limited infection; however, patients who are compromised may have a chronic infection with a wide range of symptoms (asymptomatic to severe).

Occasionally, these patients require fluid replacement and the diarrhea persists for more than 2 weeks. This is particularly true of infants, in whom excessive fluid loss

TABLE 23.2 Coccidia

Species	Shape and size	Other features
Cryptosporidium spp.	Oocyst generally round, 4–6 μm, each mature oocyst containing sporozoites	Oocyst usual diagnostic stage in stool; various other stages in life cycle can be seen in biopsy specimens taken from gastrointestinal tract (brush border of epithelial cells, intestinal tract) and other tissues (respiratory tract, biliary tract)
Cyclospora cayetanensis	Organisms generally round, 8–10 μm; they mimic *Cryptosporidium* spp. (acid fast) but are larger	In wet smears they look like nonrefractile spheres; they will also autofluoresce with epifluorescence; are acid-fast variable from no color to light pink to deep red; those that do not stain may appear wrinkled; in a trichrome-stained stool smear, they will appear as clear, round, somewhat wrinkled objects; they cause diarrhea in both immunocompetent and immunosuppressed patients
Cystoisospora (*Isospora*) *belli*	Ellipsoidal oocyst; usual range, 20–30 μm long and 10–19 μm wide; sporocysts rarely seen broken out of oocysts but measure 9–11 μm	Mature oocyst contains 2 sporocysts with 4 sporozoites each; usual diagnostic stage in feces is immature oocyst containing spherical mass of protoplasm (diarrhea, intestinal tract)
Sarcocystis hominis, *S. suihominis*, *S. bovihominis*	Oocyst thin-walled and contains 2 mature sporocysts, each containing 4 sporozoites; thin oocyst wall frequently ruptures; ovoid sporocysts each measure 9–16 μm long and 7.5–12 μm wide	Thin-walled oocyst or ovoid sporocysts occur in stool (intestinal tract)
S. "lindemanni"	Shapes and sizes of skeletal and cardiac muscle sarcocysts vary considerably	Sarcocysts contain several hundred to several thousand trophozoites, each of which measures 4–9 μm in width and 12–16 μm in length; the sarcocysts may also be divided into compartments by septa, not seen in *Toxoplasma* cysts (tissue/muscle)

may last for over 3 weeks. In general, when CD4$^+$ cells are present at levels greater than 200/μl, infections are acute and resolve in approximately 2 weeks; however, when the CD4$^+$ cell count drops below 200/μl, the infection may be chronic and may not resolve.

Based on questions that arose from the 1993 Milwaukee outbreak, regarding appropriate management and prognosis of patients with inflammatory bowel disease who are infected with *Cryptosporidium*, it is clear that cryptosporidiosis may present as an acute relapse of inflammatory bowel disease and responds to standard therapy. Antibiotics confer no benefit. It appears that immunosuppressive therapy does not predispose to chronic or severe illness in these patients. Cryptosporidiosis may present with acute findings initially mimicking Crohn's disease. The total cost of outbreak-associated illness during the Milwaukee outbreak was $96.2 million: $31.7 million in medical costs and $64.6 million in productivity losses. The average total costs for persons with mild, moderate, and severe illness were $116, $475, and $7,808, respectively. The authors also comment that these cost-of-illness estimates are conservative (10).

Failure to thrive has also been attributed to chronic cryptosporidiosis in infants. Since diarrheal illness is a major cause of morbidity and mortality in young children living in developing countries, it is likely that cryptosporidiosis plays a major role in the overall health status of these children. Apparently, cryptosporidiosis has a lasting adverse effect on linear growth (height), especially when acquired during infancy and when children are stunted before they become infected. It has also been suggested that *Cryptosporidium* may be implicated in the respiratory disease that often accompanies diarrheal illness in malnourished children. Those with respiratory infections tend to have coughing, wheezing, croup, hoarseness, and shortness of breath.

Histologically, enteric infections are characterized by villous atrophy, enlarged crypts, and infiltration of the lamina propria by inflammatory cells. Respiratory infections have been characterized by infiltration of the lamina propria by inflammatory cells, loss of cilia, hyperplasia, and hypertrophy of epithelial cells. Similar inflammatory cell infiltration has also been reported for infections involving other organs.

Immunocompromised Individuals. *AIDS (intestinal disease).* The duration and severity of diarrheal illness depend on the immune status of the patient. Most severely immunocompromised patients cannot overcome the infection, the illness becomes progressively worse with time, and the sequelae may be a major factor leading to death. The length and severity of illness may also depend on the ability to reverse the immunosuppression. In these patients, *Cryptosporidium* infections are not always confined to the gastroin-

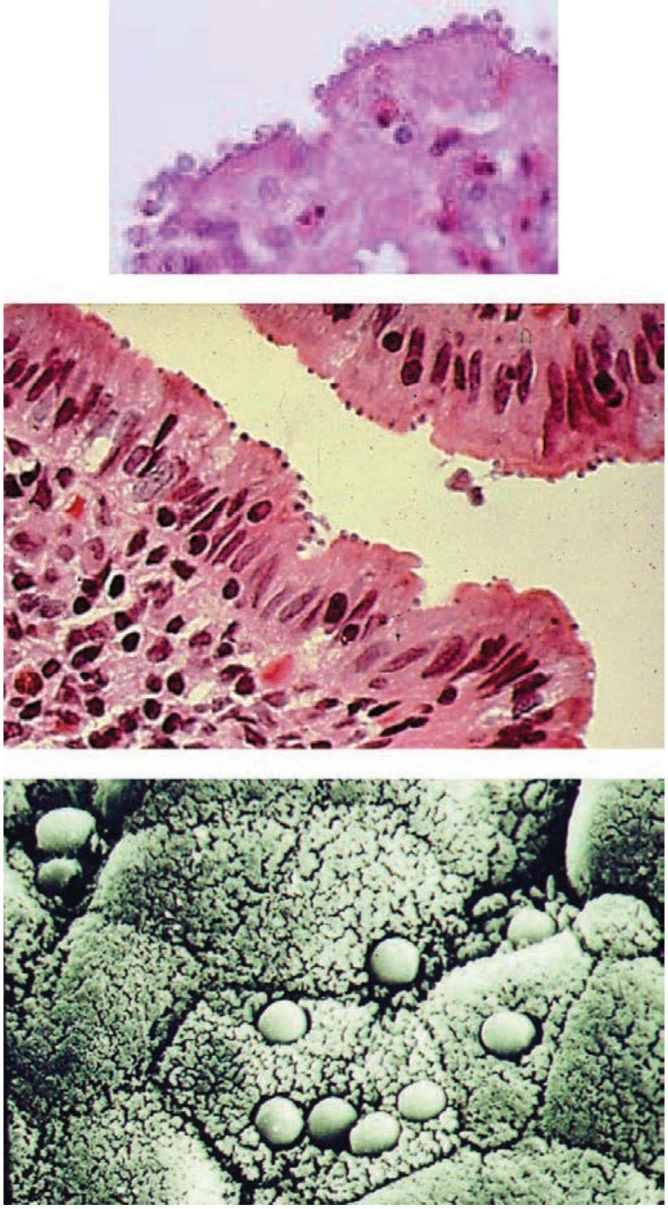


Figure 23.3 *Cryptosporidium* spp. (Top) Organisms on the brush border of the mucosal surface (courtesy of Armed Forces Institute of Pathology). (Middle) Organisms on the brush border of the mucosal surface. (Bottom) Organisms on the mucosal surface, human tissue (scanning electron micrograph, ×4,000). (Courtesy of Marietta Voge.) doi:10.1128/9781555819002.ch23.f3

testinal tract; additional symptoms (respiratory problems, cholecystitis, hepatitis, and pancreatitis) have been associated with extraintestinal infections. While the clinical features of sclerosing cholangitis secondary to opportunistic infections of the biliary tree in patients with AIDS are well known, the mechanisms by which pathogens such as *Cryptosporidium* actually cause disease are unclear.

In reviewing data from patients with gastric cryptosporidiosis, no clear correlation was found between endoscopy and histologic findings; however, a close correlation between the intensity of the infection and the degree of histologic alterations was observed (5). These findings suggest that in patients with AIDS, cryptosporidiosis, and severe immunodepression, upper endoscopy with random gastric biopsies should be performed, even in the absence of endoscopically identified lesions. The diagnosis of gastric cryptosporidiosis relies on histologic findings; pathologists need to be aware that *Cryptosporidium* organisms are found mainly in areas showing reactive hyperplasia.

When reviewing data from AIDS patients with four clinical syndromes, i.e., chronic diarrhea (36%), cholera-like disease (33%), transient diarrhea (15%), and relapsing illness (15%), there is no statistically significant correlation between the histologic intensity of infection and the clinical severity of illness. However, infected patients had a much shorter survival time than did *Cryptosporidium*-negative AIDS patients, and survival was independent of sex, race, or injection drug use (5).

Patients with CD4 counts of 180/µl or greater can usually clear the infection with *Cryptosporidium* over a period of 7 days to 1 month. These data are important in predicting the natural progression of the infection and in designing therapeutic trials. Frequent diagnosis of *Cryptosporidium* infection in patients with neoplasia and diarrhea who are undergoing chemotherapy suggests that for this patient group it is appropriate to routinely submit fecal specimens for examination for oocysts and that the cost of laboratory testing is justified.

AIDS (biliary tract disease). Sclerosing cholangitis is well known as a complication of AIDS. Direct cytopathic effects are noted in infected monolayers (human biliary epithelial cell line) and are associated with widespread programmed cell death (apoptosis) beginning within hours after exposure to the organism. This finding of specific cytopathic invasion of biliary epithelia by *Cryptosporidium* may be relevant to the pathogenesis and possible therapy of secondary sclerosing cholangitis seen in AIDS patients with biliary cryptosporidiosis. It appears that more severely immunocompromised individuals are more likely to exhibit biliary tract disease.

AIDS (pancreatitis). In AIDS patients with pancreatitis, cellular changes are generally not severe, and these patients exhibit hyperplastic squamous metaplasia. The patients usually present with abdominal pain resistant to analgesics, elevated serum amylase levels, and abnormalities on sonography and computed tomography. It is difficult to clarify the impact of cryptosporidiosis-related pancreatic disease, and this disease complication is apparently not linked to significant morbidity.

AIDS (respiratory tract disease). Because many patients with respiratory cryptosporidiosis also have other pathogens present, it is difficult to determine the significance of this complication in AIDS patients. Certainly for patients with respiratory symptoms, it is important to examine sputum as well as stool specimens to confirm the diagnosis.

Primary immunodeficiency diseases. Many different primary immunodeficiency diseases have been described; ingeneral, they can be categorized as follows: combined immunodeficiencies (which impact both T and B lymphocytes); antibody deficiencies; complement deficiencies; and defects in phagocytes (decreased number and function). In general, the number of these cases reported in relation to cryptosporidiosis is few; however, the most serious immunodeficiency in terms of risk is severe combined immunodeficiency syndrome. These patients are at risk for disseminated disease; often the prognosis is poor.

Malignant disease. Although cryptosporidiosis can be seen in patients with malignant disease, infection with *Cryptosporidium* spp. does not appear to pose a special risk. Exceptions to this general statement seem to involve leukemia and other hematologic malignancies (5). Certainly there is more interest in patients who are candidates for bone marrow transplantation, particularly when assessing possible risks related to cryptosporidiosis.

Solid-organ transplant patients. Cryptosporidiosis has been found in liver, kidney, lung, and cardiac transplant recipients, as well as following small bowel transplantation (5, 11–13). In most cases the cryptosporidiosis was not unusually severe and did not involve dissemination to extraintestinal sites. However, in children, the symptoms may be more severe; after therapy with nitazoxanide for a maximum of 21 days, all patients recovered (12).

Diagnosis

Routine. Previously, many human cases were diagnosed after examination of small or large bowel biopsy material, often under both light microscopy and EM (Fig. 23.3). However, because biopsies may miss the infected area of the mucosa, cases have recently been diagnosed by recovering oocysts from fecal material by a flotation method; several other previously reported cases were also diagnosed by using fecal concentrates (Table 23.3). Fresh fecal specimens can be concentrated by using Sheather's sugar solu-

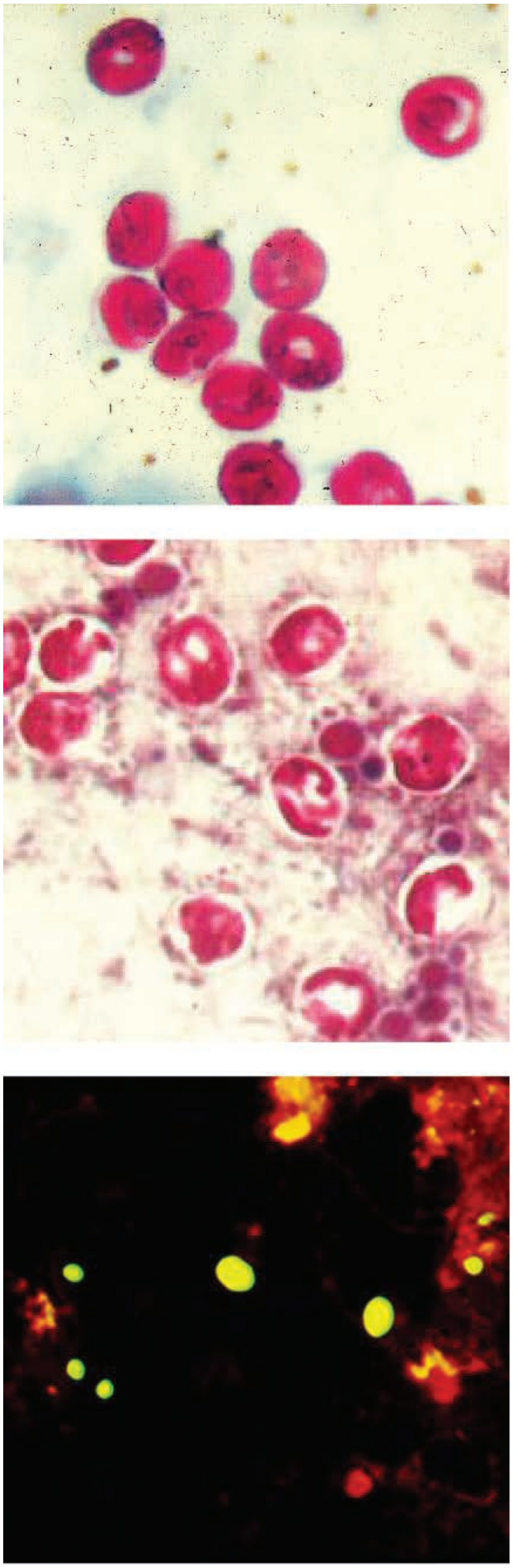


Figure 23.4 *Cryptosporidium.* (Top, middle) Oocysts from stool, stained with modified acid-fast stain. Note that in the middle image some of the sporozoites are visible within the oocyst wall. Although the sporozoites are not always seen, the oocysts are immediately infectious when passed. (Bottom) *Cryptosporidium* and *Giardia* with combination FA reagent (large organisms are *Giardia*, small organisms are *Cryptosporidium*).
doi:10.1128/9781555819002.ch23.f4

TABLE 23.3 Intestinal coccidia: recommended diagnostic procedures[a]

Organism	Specimen	Diagnostic procedure
Cryptosporidium spp.	Stool	Modified acid-fast stains, fluorescent stains, immunoassays; molecular test or instrumentation
	Sputum	Immunoassays, modified acid-fast stains
	Scraping	Modified acid-fast stains or routine histology
	Biopsy specimen	Routine histology
Cyclospora cayetanensis	Stool	Modified acid-fast stains, autofluorescence
	Biopsy specimen	Routine histology
Cystoisospora belli	Stool	Concentration sedimentation and modified acid-fast stains
	Biopsy specimen	Routine histology
Sarcocystis spp.	Stool	Concentration sedimentation and special stains
	Biopsy	Routine histology

[a] When using immunoassay reagents (FA) or modified acid-fast stains for organism detection, these procedures should be performed with centrifuged stool (500 × *g* for 10 min). When using immunoassay reagents for antigen detection (EIA, rapids), centrifugation of the fecal specimen is not required. Procedure directions must be checked, and compatibility issues between the kit reagents and stool preservatives must be kept in mind.

tion, and coverslip preparations can be examined by both phase-contrast and bright-field microscopy. The oocysts will float, and one must focus directly under the glass coverslip. Most laboratories no longer use this approach and have switched to modified acid-fast stains performed on concentrated stool sediment or the newer immunoassays (14, 16–19). For the diagnosis of cryptosporidiosis, stool and other body fluid specimens should be submitted as fresh material in 5 or 10% formalin, sodium acetate-acetic acid-formalin, or one of the newer single-vial stool collection systems. Fixed specimens are recommended because of potential biohazard considerations. Some research laboratories routinely use potassium dichromate solution (2 to 3% [wt/vol] in water) as a storage medium to preserve oocyst viability; this solution is not a fixative and is not recommended for the routine clinical laboratory. Either fresh or preserved specimens can be examined using the routine stool formalin-ethyl acetate concentration and one of the modified acid-fast stains, the fecal immunoassay tests (Table 23.4), or molecular tests (20).

Phase-Contrast Microscopy. Although examination of unpreserved flotation material by phase-contrast microscopy has proven to be an excellent procedure for the recovery and identification of *Cryptosporidium* oocysts, many laboratories have neither access to such equipment nor experience with phase-contrast microscopy. Also, since the organism is considered to be infectious to laboratory personnel, fixed specimens can be processed by using several modified acid-fast stains, many of which are very satisfactory in demonstrating the organisms in stool material (Fig. 23.4 and 23.5). The more formed and normal the specimen, the better the chance for artifact material that can be confused with *Cryptosporidium* spp. It is important to remember that the number of oocysts is directly correlated with the consistency of the stool; the more diarrheic the stool, the more oocysts are present.

Antigen Detection. The use of immunoassays has proven to be very helpful in providing a more sensitive method of detecting organisms in stool specimens. A direct fluorescent antibody (FA) procedure with excellent specificity and sensitivity has been developed and results in a significantly increased detection rate over conventional staining methods. Enzyme immunoassay (EIA) also provides excellent specificity and sensitivity for laboratories using this approach (6, 7). Some of these reagents, particularly the combination direct-FA product used to identify both *Giardia* cysts and *Cryptosporidium* oocysts, are being widely used in water testing and outbreak situations (21–27).

It is recommended that the stool specimen be centrifuged at 500 × *g* for 10 min prior to use of any of the special stains or FA immunoassay reagents; centrifugation under these conditions ensures maximum recovery of the oocysts. Centrifugation at lower speeds and for shorter times (often mentioned in some procedures) may not guarantee recovery of the oocysts. Uncentrifuged fresh, frozen, or fixed fecal material can be used for the antigen detection immunoassays; these procedures do not rely on visual identification of the oocysts (FA) but instead rely on antigen detection. The selection of fresh or frozen stool rather than fixed specimens depends on testing parameters; currently, if the test reagents or kit format includes *Entamoeba histolytica*/*E. dispar* or *E. histolytica* alone, the test format

TABLE 23.4 Commercially available kits and instruments for immunodetection of *Cryptosporidium* spp. (FDA-approved products)

Organism and kit name	Manufacturer and/or distributor[a]	Type of test	Comments
***Cryptosporidium* spp.**			The tests detect *C. hominis*, different *C. parvum* genotypes, and other species depending on the intensity of the infection
ProSpecT Cryptosporidium microplate assay	Remel (www.remel.com)	EIA	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
XPECT *Cryptosporidium* kit	Remel	Cartridge	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
Cryptosporidium antigen ELISA	Medical Chemical (www.med-chem.com)	EIA	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
Crypto-CELISA	Cellabs (www.cellabs.com)	EIA	Can be used with fresh, frozen, or formalin-preserved stool
Crypto Cel	Cellabs	DFA	
Cryptosporidium test	TechLab (www.techlabinc.com)	EIA	
Cryptosporidium test	Wampole (www.wampolelabs.com)	EIA	
Combination tests: *Cryptosporidium* and *Giardia*			
Cryptosporidium/Giardia CHEK	TechLab	EIA	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no PVA, no mercury)
ProSpecT Giardia/Cryptosporidium microplate assay	Remel	EIA	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
MERIFLUOR Cryptosporidium/Giardia	Meridian Bioscience (www.meridianbioscience.com)	DFA	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
Cryptosporidium/Giardia DFA	Medical Chemical	DFA	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
Crypto/Giardia-Cel	Cellabs	DFA	Contact manufacturer
ColorPAC Giardia/Cryptosporidium rapid assay	Becton-Dickinson (www.bd.com)	Cartridge device, lateral flow	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
ImmunoCard STAT! *Cryptosporidium/Giardia*	Meridian Bioscience	Cartridge device, lateral flow	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
Xpect Giardia/Cryptosporidium	Remel	Cartridge device, lateral flow	Can be used with fresh, frozen, or formalin-preserved stool or other single-vial systems (no formalin, no PVA, no mercury)
Combination tests: *Cryptosporidium*, *Giardia*, and *Entamoeba*			
Triage Parasite Panel	BIOSITE Diagnostics, Inc. (www.biosite.com)	Cartridge device, lateral flow	Requires fresh or frozen stool; combination test with *Giardia* and *E. histolytica/E. dispar* group; does not differentiate between *E. histolytica* and *E. dispar*

(continued)

TABLE 23.4 *(continued)*

Organism and kit name	Manufacturer and/or distributor[a]	Type of test	Comments
Molecular			
RIDAGENE Stool Panel PCR *Giardia*, *Cryptosporidium*, *E. histolytica*, *Dientamoeba*	R-Biopharm AG, Darmstadt, Germany	PCR	Panel can be used with multiple instruments
xTAG Gastrointestinal Pathogen Panel (xTAG CPP), viral, bacterial, parasitic pathogens (*Giardia*, *Cryptosporidium*, *E. histolytica*)	Luminex, Austin, TX	PCR	Multiplexed molecular panel for bacterial, viral, and parasitic organisms; fresh or frozen stool (unpreserved)
BD MAX Enteric Parasite Panel (*Giardia*, *Cryptosporidium*, *E. histolytica*)	Becton, Dickinson and Co., Sparks, MD	PCR	Unpreserved or 10% formalin-fixed stools
BioFire FilmArray Gastrointestinal Panel, bacterial, viral, parasitic pathogens (*Cryptosporidium*, *Cyclospora*, *E. histolytica*, *Giardia*)	bioMérieux, Marcy l'Etoile, France	PCR	Multiplex molecular panel; stool in Cary Blair

[a] URLs are given only the first time the company appears in the table.

requires fresh or frozen stool. If the test format includes *Cryptosporidium* and/or *Giardia*, fresh, frozen, *or* preserved stools can be used. *However, it is always important to check with the manufacturer to confirm that the particular stool fixative you are currently using will be compatible with the immunoassay test reagents.* It is also important to review laboratory workflow and test request numbers prior to making test method selections (EIA, FA, rapid cartridge). Multiple stool specimens may have to be examined to diagnose the infection; this is particularly true when dealing with formed stool specimens, which may have low organism numbers.

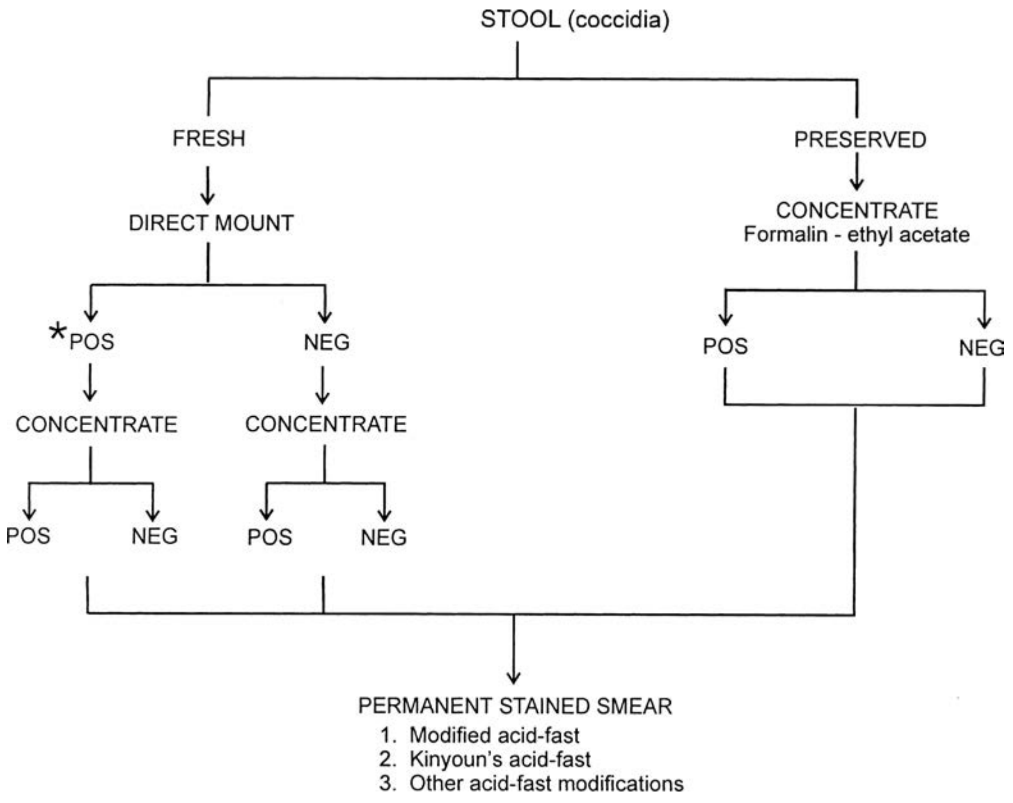


Figure 23.5 Diagnostic work flow diagram leading from submission of stool specimen to final permanent-stained smear for *Cryptosporidium* spp. and *Cyclospora cayetanensis*. *, Identification of coccidia may be difficult in a direct wet mount unless there are numerous oocysts present. In many laboratories, the use of fecal immunoassays has replaced acid-fast staining techniques for *Cryptosporidium* spp. The immunoassays can be performed on unconcentrated material (EIA, lateral-flow rapid cartridge) or concentrated material (FA assay). doi:10.1128/9781555819002.ch23.f5

Ordering/Testing Options. Various screening approaches have been used, and the approach varies from testing all specimens submitted for a routine stool examination to testing limited risk groups or testing only on request. There are numerous pros and cons to each approach; each laboratory will have to select its own approach on the basis of the patient base, client preferences, single versus batch testing, opportunity for in-service education for clients, availability of personnel, level of personnel training and expertise, clinical relevance, and cost. Prevalence data tend to be low unless specific risk groups are screened. A comprehensive approach would be to screen all symptomatic patients; however, even this approach often results in the identification of very few positive patients and is certainly not particularly cost-effective. A selective approach involving all compromised patients or compromised patients with diarrhea might be more reasonable. However, this assumes that the laboratory will always have access to this type of patient information, an assumption that is not realistic. Many laboratories have elected to provide in-service education to their physician clients and will perform these procedures only on request. Options for test ordering can be seen in Table 23.5. Although there is evidence for regional and seasonal variability of cryptosporidiosis, these possibilities will probably have little impact on the laboratory's overall approach to diagnostic testing.

Respiratory Cryptosporidiosis. Respiratory cryptosporidiosis has been found in AIDS patients. Sputum specimens from immunodeficient patients with undiagnosed respiratory illness should be submitted in 10% formalin and examined for *Cryptosporidium* oocysts by the same techniques as used for stool samples. Infection of the gallbladder and biliary tree should result in oocysts being passed in the stool.

Antibody Detection. Preliminary results from serologic methods for the diagnosis of cryptosporidiosis appear promising; however, additional patients must be studied

TABLE 23.5 Testing options for the diagnosis of cryptosporidiosis[a]

History	Pros	Cons
No travel outside United States		
Test every stool		
Modified acid-fast stain	Identifies infected patients	May not detect carriers or patients with small number of organisms
Immunoassay	Is more sensitive than the modified acid-fast stains and may identify patients who are carriers with low parasite numbers	High cost; may not be clinically relevant to screen all patients, particularly if there are small numbers in the patient base
Test on order only (after appropriate education of the physician client base)		
Geographic area with little to no reported cases	Is clinically relevant and more cost-effective	Still necessary to set up linkages with public health, water companies, etc., so that appropriate communication can serve as a link and notice of possible early outbreak situation
Geographic area with reported cases and history of outbreak situations	Education of the physician client base targets potential patient risk groups and potential problem situations	Still necessary to continue linkages with public health, water companies, etc., so that appropriate communication can serve as a link and notice of possible early outbreak situation
Travel outside United States		
Order routine ova and parasite examinations (minimum of two; must include the concentration and permanent stained smear)	Facilitates detection and identification of possible other relevant pathogens. If patient has become asymptomatic by the time the ova and parasite examination results are available (negative results), no additional testing is required.	Routine ova and parasite exam will not reveal the presence of *Cryptosporidium* (with rare exceptions based on organism numbers and method selection). If the patient remains symptomatic after the ova and parasite examination results (negative results) are available, more sensitive immunoassay procedures can be performed.
Order routine ova and parasite examinations and immunoassay for *Cryptosporidium*	This approach may be more clinically relevant in terms of turnaround time issues.	May result in excess testing

[a] It is important to remember that laboratories in different geographic regions may approach this testing differently. The important point is to ensure that physicians and laboratory personnel communicate regarding the pros and cons of each approach. Preliminary education, selection of the most clinically relevant approach, client education and communication, and periodic review of testing options and approaches will result in more cost-effective and better patient care.

before the actual diagnostic potential of these methods can be evaluated. Serologic tests have been very useful in estimating the prevalence of the infection in different parts of the world. Some of the data reveal very interesting associations between seroprevalence, outbreak-related laboratory serologic data, and patterns of parasite contamination of drinking water (21).

Flow Cytometry. Flow cytometry methods for the quantitation of *Cryptosporidium* oocysts in stool specimens have been developed as an alternative approach. Studies indicate that the results are approximately 10 times more sensitive than those of conventional immunofluorescence assays. However, this approach is somewhat impractical for most clinical laboratories.

Molecular Testing. PCR technology also offers alternatives for the diagnosis of *Cryptosporidium* in both clinical and environmental samples. Compared with microscopic examination by conventional acid-fast staining procedures, PCR is more sensitive and easier to interpret but requires more "hands-on" time and expertise, as well as being more expensive. An important advantage of PCR is the ability to directly differentiate between different *Cryptosporidium* genotypes, which is important in outbreak situations. Sensitivity, specificity, ability to genotype, ease of use, and adaptability to batch testing make PCR a useful tool for future diagnostic and molecular epidemiologic studies.

A 19plex laboratory-developed gastrointestinal pathogen panel using Luminex xTAG analyte-specific reagents has been validated, which simultaneously screens directly in fecal specimens for diarrhea-causing pathogens, including bacteria, viruses, and parasites (*Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*). Overall sensitivity was 94.5% with a specificity of 99.8% (20).

KEY POINTS—LABORATORY DIAGNOSIS

***Cryptosporidium* spp.**

1. If the specimen represents the typically watery diarrhea, numerous organisms should be caught up in mucus.
2. The more normal the stool (semiformed, formed), the fewer the organisms and the more artifact material that will be present. It is unlikely that the oocysts will be seen in a wet preparation unless the parasite load is quite heavy; in this case, suspicious objects seen in the wet mount may be suggestive of coccidian oocysts.
3. Several concentrates and at least five or six modified acid-fast smears should be examined before a patient is considered negative, especially if the patient has been receiving experimental medications.
4. The normal permanent stains (trichrome, iron hematoxylin) do not adequately stain *Cryptosporidium* spp., although oocysts are occasionally seen in these preparations, especially those from patients with heavy infections.
5. Although the organisms can be stained with auramine-rhodamine stains, they should be confirmed by using acid-fast stains or the immunoassay reagents (most sensitive). This is particularly true if the stool contains other cells or a lot of artifact material (more normal stool consistency).
6. When using immunoassay reagents, it is important to remember that the stool specimen fixative and immunoassay reagents must be compatible. Concentrated stool sediment (500 × *g* for 10 min) should always be used for staining tests (modified acid-fast, etc.) and FA immunoassay because the tests are detecting the oocysts. However, when immunoassay reagents are used to detect *Cryptosporidium* antigen, the specimen does not have to be concentrated before being tested. Check specific kit directions and chapter 33.
7. Sputum specimens should be submitted in 5 or 10% formalin and processed in the same way as a stool sample.
8. It is important to remember that microsporidia are also present in approximately 30% of severely immunocompromised patients (<100 CD4$^+$ cells) who have cryptosporidiosis. The diagnostic procedures for the identification of *Cryptosporidium* spp. are not appropriate for the identification of microsporidial spores. Modified trichrome stains and optical brightening agents (calcofluor white) can be used for that purpose (17).

Histology. When examining histology preparations, developmental stages in the life cycle of *Cryptosporidium* spp. can be found at all levels of the intestinal tract, with the jejunum being the most heavily infected site. Routine hematoxylin and eosin staining is sufficient to demonstrate these parasites. Under regular light microscopy, the organisms are visible as small (~1 to 3 μm), round structures aligned along the brush border. They are intracellular but extracytoplasmic and are found in parasitophorous vacuoles. Developmental stages are more difficult to identify without using transmission EM. It is also important to remember that *Cryptosporidium* spp. have been found in other body sites, primarily the lungs, as a disseminated infection in severely immunocompromised patients. Within tissue, confusion with *Cyclospora cayetanensis* is unlikely, because the oocysts of this coccidian are approximately 8 to 10 μm and the developmental stages occur within a vacuole at the luminal end of the enterocyte, rather than at the brush border. Developmental stages of *Cystoisospora belli* also occur within the enterocyte and so should not be confused with *Cryptosporidium* spp.

Newer methods involving PCR have also been used to detect *Cryptosporidium* DNA in fixed, paraffin-embedded tissue. This approach could provide a sensitive and specific method for detection of parasite material in paraffin-embedded tissues and could be very valuable in retrospective studies of archival material. PCR has also been used to detect viable *Cryptosporidium* oocysts. Using PCR, confirmation of the presence of *Cryptosporidium* spp. in environmental studies has become more widely used (27–32).

Water Quality and Testing. During the past few years, the issues of water quality and water testing have become more important and controversial. Since the late 1980s, most of the water utilities have either set up in-house water quality testing laboratories or contracted with commercial water laboratories for the recovery and identification of *Cryptosporidium* and *Giardia* species. In a recent study, two methods were evaluated for the recovery of *Cryptosporidium* and *Giardia* species. The American Society for Testing and Materials method involves sampling a minimum of 100 liters of water through a polypropylene yard cartridge filter, extracting the particulates, flotation-concentrating the extracted particulates on a Percoll-sucrose gradient with the commercially available *Cryptosporidium*/*Giardia* combination FA reagent, and reviewing the slides for the presence of *Cryptosporidium* oocysts and/or *Giardia* cysts. This method tends to be labor-intensive, complex, and lengthy and depends on the skill and experience of the personnel and the quality control measures in use within the laboratory. Recovery rates tend to be low with this approach. The second method uses sampling by membrane filtration, Percoll-Percoll step gradient, and immunofluorescent staining; this method was characterized by higher recovery rates in all three types of waters tested: raw surface water, partially treated water from a flocculation basin, and filtered water. When seeded specimens were used, much higher recovery rates were obtained when the flotation step was eliminated from the protocol. Oocyst and cyst recovery rates decreased in both methods as the turbidity of the water increased. When smaller tubes were used for flotation, the membrane filter method was less time-consuming and cheaper. However, the lack of a confirmatory step might be a problem, particularly if cross-reacting algae are present in water samples. A number of other test options, including PCR, are currently being used (22–32). Unfortunately, these procedures emphasize organism detection; the issues of organism viability and infectivity have not yet been totally resolved.

Treatment

Cryptosporidiosis tends to be self-limiting in patients who have an intact immune system. For patients who are receiving immunosuppressive agents, one method of therapy would be to discontinue such a regimen. Other approaches with specific therapeutic drugs have been tried, but to date the results are still somewhat controversial (Table 23.6). Tissue culture systems with cell lines such as human ileocecal adenocarcinoma (HCT-8) cells and animal models that support the entire life cycle have been developed, and these may provide excellent opportunities for *in vitro* drug studies.

Immunocompetent Patients. Currently, the drug of choice for immunocompetent individuals is nitazoxanide. Studies indicate a statistically significant reduction in median oocyst excretion after 2 weeks of therapy with paromomycin. Several studies have reported success for a combination of paromomycin (1 g twice daily) and azithromycin (600 mg daily) for 4 weeks followed by paromomycin monotherapy for an additional 8 weeks. Recommended dosages can be found in chapter 36 (33).

Immunocompromised Patients. No drug has proven to be effective in this patient population. Ongoing studies with immunologic intervention hold promise, but this approach is still being evaluated in clinical trials. One approach that has had a dramatic impact on cryptosporidiosis in AIDS patients is highly active antiretroviral therapy (HAART) leading to an increased CD4 count. Resolution of the diarrhea resulting from cryptosporidiosis is apparently related to the enhanced CD4 count rather than to any change in the viral load or any therapeutic impact of the drugs themselves. Thus, it appears that cellular immunity is critical in clearing *Cryptosporidium* infection in these patients. Nitazoxanide (treatment duration of 5 to 21 days), paromomycin, or a combination of paromomycin and azithromycin may be tried to decrease diarrhea and continued malabsorption of antimicrobial drugs, which can occur with chronic cryptosporidiosis.

Treatment Summary. Although over 100 therapeutic agents have been evaluated, nitazoxanide is the only drug that has been approved by the FDA for treatment of cryptosporidiosis in immunocompetent persons in the United States. However, this drug is not effective in immunocompromised individuals. In patients with HIV/AIDS, the mainstay of treatment is immune reconstitution with HAART, which results in clearance or abrogation of infection (33). There is no vaccine available for cryptosporidiosis.

Epidemiology and Prevention

Epidemiology. Information on the epidemiology of *Cryptosporidium* continues to expand, since its potential pathogenicity in humans is now well documented (Tables 23.7 to 23.9; Fig. 23.6 to 23.8). The organism is transmitted

TABLE 23.6 Chemotherapeutic agents and supportive therapy for the treatment of human cryptosporidiosis[a]

Chemotherapeutic agent[b]	Comments
Recommended agents	
Nitazoxanide	This is a nitrothiazole benzamide with a wide spectrum of activity against coccidia, amebae, nematodes, cestodes, and trematodes. Preliminary data appear promising, with suggested activity against cryptosporidial infection in AIDS patients. The agent appears to be well tolerated. Additional trials are under way. Nitazoxanide is FDA-approved only for treatment of diarrhea caused by *Giardia* or *Cryptosporidium*[c]. It is available in 500-mg tablets and an oral suspension and should be taken with food.
Paromomycin (Humatin)	Paromomycin is synonymous with aminosidine (marketed outside the United States). It is an oligosaccharide aminoglycoside related to kanamycin; it achieves high concentrations in the colon but is poorly absorbed. Studies indicate that the drug effect is probably static rather than cidal for *Cryptosporidium*. Although decreased bowel movements and oocyst shedding have been reported, a small number of patients developed biliary tract disease and some of these required cholecystectomy. Despite conflicting results, paromomycin currently is used as the first-line drug in treating cryptosporidiosis. Since patients enter treatment trials after paromomycin failure, the question of delay and possible spread to the biliary tree has been raised.
Macrolides	
Spiramycin (oral)	Although a number of studies have been reported, divergent results are seen. Placebo results were often comparable to those obtained from patients receiving the drug. Absorption seems to be decreased in the presence of food. Decrease in diarrhea and oocyst excretion was seen in immunocompetent infants randomized to receive spiramycin (100 mg/kg/day) or placebo. A positive clinical response, as well as organism eradication, has also been reported in a group of AIDS patients.
Spiramycin (intravenous)	This approach was taken to circumvent absorption problems. Data revealed a statistically significant drop in oocyst count compared to placebo; however, administration of intravenous spiramycin was associated with paresthesias, taste perversion, nausea, and vomiting. At doses of >75 mg/kg/day there were cases of severe colitis due to intestinal injury.
Azithromycin	This drug has a long half-life (6 to 8 h) and achieves high concentrations in tissue, particularly in the biliary tree and gallbladder. Although decreased oocyst counts have been obtained from patients with high drug levels in serum, marginal improvement has been seen in other patients. A lactose-free form of azithromycin in 300-mg tablets is available through Pfizer's compassionate-use program. There may be a therapeutic effect on biliary tree infection, particularly in patients receiving intravenous azithromycin. Intravenous azithromycin at doses up to 2 g daily for 2 weeks is well tolerated.
Clarithromycin	Little information is available on other macrolides, including clarithromycin, roxithromycin, and dirithromycin; however, studies are in progress.
Benzeneacetonitrile derivatives	
Diclazuril	Interest stems from its efficacy for *Eimeria*; however, reports indicate that doses ranging from 50 to 800 mg were no more efficacious than placebo. This may have been due to poor drug bioavailability.
Letrazuril	Poor absorption of diclazuril led to the development of letrazuril. Although bioavailability was improved, patient clinical improvement was minimal. Data also suggest that this drug may interfere with modified acid-fast staining methods.
Miscellaneous agents	
Difluoromethylornithine (DFMO) (Eflornithine)	Although some success with this agent against *Cryptosporidium* in AIDS patients has been reported, serious toxicity has been documented (bone marrow suppression, gastrointestinal intolerance, and hearing impairment).
Atovaquone (Mepron)	This drug is an antimalarial that has been evaluated against *Pneumocystis jirovecii*, *Toxoplasma gondii*, and *Cryptosporidium* in AIDS patients. Preliminary results do not look promising.
Supportive therapeutic agents	
Nonspecific antidiarrheal agents (Kaopectate, Imodium, Lomotil, Pepto-Bismol, opiates)	These agents are often helpful, but individual regimens need to be developed for each patient; safety in patients with cryptosporidiosis is not known.
Octreotide (Sandostatin)	This is a synthetic cyclic octapeptide analog of somatostatin; anecdotal reports indicate some success in treating AIDS-related diarrhea. However, most patients had neither cryptosporidial diarrhea nor other identifiable pathogens.

[a] Adapted from Blagburn BL, Soave R, *in* Fayer R (ed), Cryptosporidium *and Cryptosporidiosis*, ASM Press, Washington, DC, 1997, and reference 33.

[b] Although other agents have been tried (zidovudine, recombinant interleukin-2), any improvement is most probably due to increased patient immune function rather than to the antiparasitic activity of the drug.

[c] *Med Lett Drugs Ther* **45**:29, 2003.

TABLE 23.7 Biological factors which impact the epidemiology of *Cryptosporidium*[a]

- Small (4–6 µm), environmentally resistant, and fully sporulated/infectious oocysts when passed; although sporozoites may not be seen in every oocyst, they are immediately infectious when passed
- Large livestock animal and human reservoir host populations
- Ubiquitous, able to cross-infect multiple host species (not species specific)
- Low infective dose (≤10–100 oocysts)
- Large multiplication capability ($>10^{10}$) in a single host
- Resistance to disinfectants
- Lack of effective therapy; resistance to available drugs

[a] Adapted from Casemore DP et al., *in* Fayer R (ed), Cryptosporidium *and Cryptosporidiosis*, ASM Press, Washington, DC, 1997.

by oocysts that are normally fully sporulated and infective at the time they are passed in stool. The oocysts are resistant to some of the disinfectants that are routinely used in medical care facilities. Exactly which of the oocyst layers is primarily responsible for the mechanical rigidity and resistance to disruption exhibited by the intact oocyst is not known. However, it has been suggested that the central glycolipid/lipoprotein layer and the inner filamentous layer may provide rigidity and elasticity to the wall.

TABLE 23.8 Risk factors for acquisition of cryptosporidiosis

Risk factor	Examples
Deficient immunity	AIDS, other acquired or congenital immunodepression syndromes (e.g., boys with mutation of the CD154 gene, congenital X-linked immunodeficiency with hyper-IgM), immunosuppression, malnutrition
Zoonotic contact	Leisure activities such as camping, backpacking, farm visits
Occupational exposure	Veterinary, agricultural, nursing or medical, laboratory, day care centers
Poor sanitary conditions	Drinking water and food, inadequate sewage or waste disposal, inadequate fly control
Exposure to untreated water (surface or recreational)	Leisure activities
Exposure to inadequately treated water supply	Inadequate treatment or breakdown in treatment process
Consumption of raw foods	Unpasteurized milk, raw meat
Travel	Travel from developed to underdeveloped countries and from urban to rural areas
Age (infants, young children)	Weaning, teething, pica, finger sucking, wearing diapers; poor infection control
Contact with case of diarrhea	Day care, household, parents

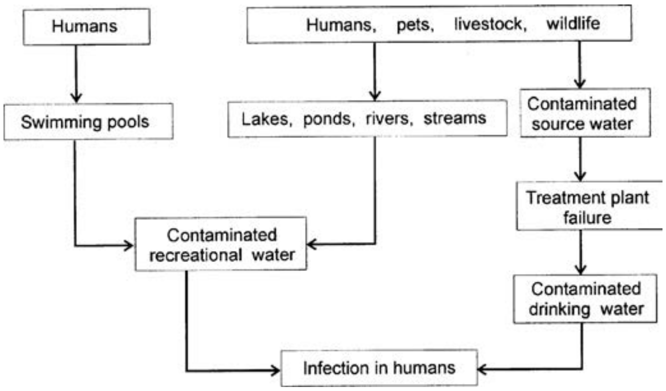
TABLE 23.9 Factors related to potential outbreaks of cryptosporidiosis

Factor	Examples
Factors unrelated to water	Sufficient number of parasites (viability, infectivity, virulence) Sufficient number of susceptible individuals for ingestion of oocysts leading to primary infection Sufficient number of susceptible individuals for whom contact with primary case leads to secondary transmission Adequate epidemiologic and laboratory surveillance and reporting
Water-related factors	Abnormal weather conditions (heavy rainfall); increased load for water treatment facilities High turbidity of surface water; increased challenge for water treatment facilities Breakdown in integrity of groundwater aquifers (prolonged rain after drought) Suboptimal water treatment functions Problems with finished-water distribution

Studies have shown that calves and perhaps other animals serve as potential sources of human infections. Contact with these animals may be an unrecognized cause of gastroenteritis in humans in both rural and urban settings. Direct person-to-person transmission is also likely and may occur through direct or indirect contact with stool material. Direct transmission may occur during sexual practices involving oral-anal contact. Indirect transmission may occur through exposure to positive specimens in a laboratory setting or from contaminated surfaces or food or water (10, 14, 21–29, 30–32, 37, 39).

Agricultural wastewater and runoff, particularly from lambs and calves, is also now recognized as an important potential source of infective *Cryptosporidium* oocysts (27). It has been shown that in healthy adults with no serologic

Figure 23.6 Flow diagram demonstrating sources of waterborne transmission of *Cryptosporidium* oocysts. doi:10.1128/9781555819002.ch23.f6


Humans
Humans, pets, livestock, wildlife
Swimming pools
Lakes, ponds, rivers, streams
Contaminated source water
Treatment plant failure
Contaminated recreational water
Contaminated drinking water
Infection in humans

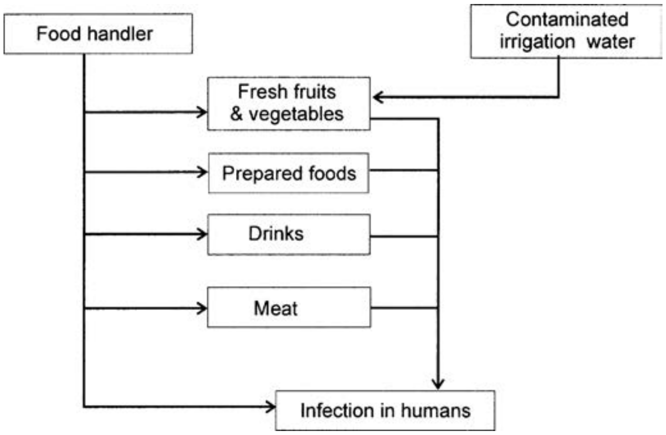


Figure 23.7 Flow diagram demonstrating sources of foodborne transmission of *Cryptosporidium* oocysts.
doi:10.1128/9781555819002.ch23.f7

evidence of past infection with *Cryptosporidium*, a low dose of oocysts is sufficient to cause infection. With healthy human volunteers, the median infective dose was 132 oocysts, and one-fifth of the volunteers became infected with a dose as low as 30 oocysts. Specific guidelines are available for the prevention of cryptosporidiosis, particularly where drinking water is involved. Information can be seen in Tables 23.9 to 23.16.

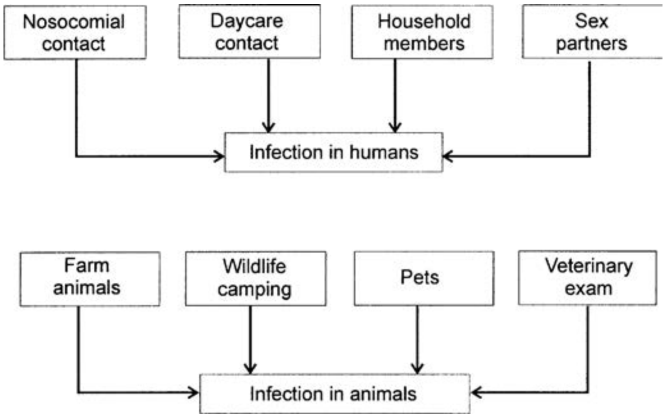
TABLE 23.10 Physical disinfection of *Cryptosporidium* oocysts[a]

Agent	Conditions	Results
Heat	50–55°C, 5 min	Noninfectious (*in vivo*, mice)
	45°C, 20 min	Noninfectious (*in vivo*, mice)
	60°C, 6 min	Noninfectious (*in vivo*, mice)
	59.7°C, 5 min	Infectious (*in vivo*, mice)
	64.2°C, 5 min	Noninfectious (*in vivo*, mice)
	67.5°C, 1 min	Infectious (*in vivo*, mice)
	72.4°C, 1 min	Noninfectious (*in vivo*, mice)
Freezing	−196°C, 10 min	Noninfectious (*in vivo*, mice)
	−70°C, 1 h	Noninfectious (*in vivo*, mice)
	−20°C, 1 day,	Noninfectious (*in vivo*, mice)
	3 days	Infectious (*in vivo*, mice)
	−20°C, 8 h	Noninfectious (*in vivo*, mice)
	−15°C, 1 wk	Infectious (*in vivo*, mice)
	−15°C, 24 h	Infectious (*in vivo*, mice)
	−10°C, 1 wk	100% reduced (excystation, dyes)
	Liquid nitrogen	98% reduced (excystation, dyes)
	−22°C, 32 days	
UV light	15,000 mW/s, 2 h	Infectious (*in vivo*, mice)
	15,000 mW/s, 2.5 h	Noninfectious (*in vivo*, mice)
	80 mW/s cm^{-2}	90% reduced (excystation)
	120 mW/s cm^{-2}	99% reduced (excystation)
	8,748 mW/s cm^{-2}	100% reduced (excystation, dyes)
Drying	Air dried, 2 h	97% reduced (excystation, dyes)
	Air dried, 4 h	100% reduced (excystation, dyes)
	Air dried in feces, 1–4 days	Noninfectious (*in vivo*, mice)

[a]Adapted from Fayer R et al *in* Fayer R (ed), Cryptosporidium *and Cryptosporidiosis,* ASM Press, Washington, DC, 1997.

Various surveys indicate prevalence rates of 1 to 2% in Europe, 0.6 to 4.3% in North America, and 3 to 20% in other areas of the world (Asia, Australia, Africa, and Central and South America). It has been suggested that the infection rate for individuals with diarrheal illness is 2.2% for persons living in industrialized countries and 8.5% for those residing in developing countries. Available data indicate that there are 250 million to 500 million *Cryptosporidium* infections annually among persons living in Asia, Africa, and Latin America. Generally, young children tend to have higher infection rates and all patients appear to have symptoms. Available serologic data support the prevalence rates, with figures from Europe and North America normally between 25 and 35% and those from South America indicating rates of up to 64%.

Figure 23.8 Flow diagram demonstrating sources of close-contact transmission of *Cryptosporidium* oocysts.
doi:10.1128/9781555819002.ch23.f8



The epidemiologic considerations for cryptosporidiosis emphasize transmission by environmentally resistant oocysts, numerous potential reservoir hosts for zoonotic transmission, documentation of person-to-person transmission in day care centers, nosocomial transmission within the health care setting, occurrence of asymptomatic infections (infective, carrier state), widespread environmental distribution resulting in the probability of waterborne transmission, and the link between cryptosporidiosis and severe, life-threatening disease in individuals with impaired immune function. Prevalence data from the Centers for Disease Control and Prevention for patients with AIDS are thought to be an underestimate of the actual prevalence (2 to 5%), particularly when other figures indicate percentages of 15% (National Institutes of Health, Bethesda, MD) and 11% (Great Britain). Surveillance systems currently under discussion can be seen in Table 23.15.

As our understanding of the epidemiology of cryptosporidiosis expands, it is obvious that there are numerous

TABLE 23.11 Waterborne outbreaks within the United States associated with water intended for drinking[a]

	No. of outbreaks (no. of cases) in:										
Parasite	**1984**	**1985**	**1986–1988**	**1989–1990**	**1991–1992**	**1993–1994**	**1995–1996**	**1997–1998**	**1999–2000**	**2001–2002**	**Total[b]**
Cryptosporidium spp.	1 (117)		1 (13,000)		3 (3,551)	5 (403,246)		2 (1,4001)	1 (5)	1 (10)	14 (421,329)
C. cayetanensis				1 (21)		2 (23)					3 (44)
G. lamblia	6 (879)	3 (741)	9 (1,169)	7 (697)	4 (123)	5 (385)	2 (1,460)	4 (159)	6 (52)	3 (18)	49 (5,683)
E. histolytica	1 (4)										1 (4)

[a] Data from the *CDC Surveillance for Waterborne-Disease Outbreaks—United States* (usually provided on a 2-year cycle with a 2- to 4-year delay).

[b] In June/July 2004, approximately 50 people were infected with *C. cayetanensis* from the ingestion of snow peas (7). In 2000, there were 1,368 cases of cryptosporidiosis with 13 outbreaks associated with recreational water (data from *CDC Surveillance for Waterborne-Disease Outbreaks—United States [1999–2000]* **51:**SS-8, 2002). In 2009–2010, there were 27 outbreaks with 436 cases of cryptosporidiosis associated with recreational water (data from *CDC Recreational Water-Associated Disease Outbreaks—United States [2009–2010]* **63:**6–10, 2014).

ways for the resistant oocysts to become disseminated. Some of these recent examples have generated enhanced interest in and concern about additional sources of infectious oocysts and the potential for accidental human consumption through contaminated food and/or water. Specific examples can be seen in Table 23.16 and Fig. 23.6, 23.7, and 23.8.

Prevention. Oocysts are susceptible to ammonia, 10% formalin in saline, freeze-drying, and exposure to temperatures above 65°C for 30 min. Commercial bleach in a 50% solution is also effective (Table 23.10). For most of the chemicals that have been tried for disinfection, effective concentrations are not practical outside the laboratory and high concentrations that significantly reduce oocyst infec-

TABLE 23.12 Prevention of cryptosporidiosis: recommendations (particularly important for patients who are immunocompromised)[a]

Preventive measure	Comments
Handwashing	Prior to eating and food preparation; after touching children in diapers; after touching clothing, bedding, toilets, bed pans from anyone with diarrhea; after gardening; after touching pets or other animals; after touching or coming in contact with anything contaminated with human or animal stool (includes dirt); after removing gloves to perform any of the above activities.
Practicing safe sex	Always wash hands after touching partner's anal or rectal area.
Avoiding touching farm animals	Particularly important for contact with young animals; wash hands thoroughly; avoid touching stool of any animal; after potential exposure, clean shoes (HIV-infected persons should not perform this task).
Avoiding pets' stools	If HIV infected, avoid cleaning litter boxes or cages or stool disposal (very important if animals are younger than 6 months); have puppies or kittens (if younger than 6 months) tested for presence of *Cryptosporidium* before bringing them home; have any pet tested that has diarrhea.
Swimming	Avoid swallowing water when in lakes, rivers, pools, or hot tubs; organisms are not killed by routine chlorination; avoid swimming in polluted ocean water, since oocysts can survive for several days in salt water.
Washing and cooking food	Thoroughly wash all fruits and vegetables if eating uncooked; use safe water (see below) for washing food; peel fruit; avoid unpasteurized milk or dairy products. Cooking kills *Cryptosporidium,* so cooked or packaged food is safe unless handled by someone infected with the organism.
Drinking water	Do not drink directly from lakes, rivers, streams, or springs; may want to avoid tap water (including refrigerator ice-maker ice and chilled water); boil water (rolling boil for 1 min is sufficient to kill organisms); filter with appropriate filters (look for "reverse osmosis," "absolute 1 micron," "Standard 53," and the words "cyst reduction" or "cyst removal"); a home distiller can be used. Store filtered water as for boiled water.
Bottled water	Bottled water labels reading "well water," "artesian well water," "spring water," or "mineral water" do not guarantee that the water does not contain *Cryptosporidium.* However, water that comes from protected well or spring water sources is less likely to be contaminated than bottled water from rivers and lakes.
Drinks	Canned or bottled soda, seltzer, fruit drinks, and steaming hot tea and coffee are safe; fountain drinks, fruit drinks from concentrate mixed with tap water, and iced tea or coffee may not be safe.

[a] Adapted from information prepared by the Interagency Working Group on Waterborne Cryptosporidiosis.

TABLE 23.13 Filter options for removal of *Cryptosporidium* oocysts: label information[a]

Label information for filters designed to remove *Cryptosporidium*[b]	Label information for filters that may not be designed for removal of *Cryptosporidium*
Reverse osmosis (with or without NSF testing)	*Nominal* pore size of “1 micron” (1 µm) or smaller
Absolute pore size of “1 micron” (1 µm) or smaller (with or without NSF testing)	“One-micron” (1 µm) filter Effective against *Giardia* Effective against parasites
Tested and certified by NSF Standard 53 for cyst removal Tested and certified by NSF Standard 53 for cyst reduction	Carbon filter Water purifier EPA approved (*Caution*: EPA does not approve or test filters) EPA registered (*Caution*: EPA does not register filters for *Cryptosporidium* removal) Activated carbon Removes chlorine Ultraviolet light Pentiodide resins Water softener

[a] Adapted from information prepared by the Interagency Working Group on Waterborne Cryptosporidiosis.

[b] Any of the four comments in this column on a package label will indicate that the filter should be able to remove *Cryptosporidium*.

tivity are either very expensive or quite toxic. Unfortunately, oocysts of *Cryptosporidium* have shown high levels of resistance to most commercial disinfectants, including iodine water purification tablets (34). Although chlorine and related compounds can dramatically reduce the ability of oocysts to excyst or infect, high concentrations and long exposure times are required, making this approach impractical. Although rarely used in routine clinical laboratories, stool specimens can be collected in potassium dichromate specifically for *Cryptosporidium* spp., and the oocysts remain viable in this solution for at least 12 months. In general, it is preferred that the oocysts be rendered noninfectious prior to submission to the laboratory for diagnostic testing.

TABLE 23.14 Bottled water: label information[a]

Label information for bottled water that has been processed by a method effective for removal of *Cryptosporidium*	Label information for bottled water that may not have been processed by a method effective for removal of *Cryptosporidium*
Reverse osmosis treated	Filtered
Distilled	Microfiltered
Filtered through an *absolute* “1 micron” (1 µm) or smaller filter	Carbon filtered Particle filtered
“One micron” (1 µm) *absolute*	Multimedia filtered
	Ozonated
	Ozone treated
	Ultraviolet light treated
	Activated carbon treated
	Carbon dioxide treated
	Ion exchange treated
	Deionized
	Purified
	Chlorinated

[a] Adapted from information prepared by the Interagency Working Group on Waterborne Cryptosporidiosis.

Almost all oocysts exposed to soils that are frozen at −10°C become inactivated within 50 days; inactivation occurs regardless of the presence or absence of freeze-thaw cycles (35). Although oocysts that are purified from fecal material prior to cryopreservation tend to lose viability and infectivity for tissue culture and mice, when the oocysts are cryopreserved in feces a certain number remain viable and maintain infectivity. Based on these studies, fecal material appears to protect the oocysts during cryopreservation stored at −20°C for at least 30 days (36).

Outbreaks. In January and February 1987, cryptosporidiosis was associated with an estimated 13,000 cases of gastroenteritis in Carroll County, GA. *Cryptosporidium* oocysts were identified in the stools of 39% of the persons examined during the outbreak; a randomized telephone survey indicated probable attack rates of 54% within the city of Carrollton and 40% overall for the county. The only significant risk factor associated with illness was exposure to the public water supply, which was filtered and chlorinated. It was interesting that, according to records kept during the outbreak, the treatment facility was operating within Environmental Protection Agency guidelines. Additional reports of waterborne outbreaks have appeared in the past few years (Table 23.11). Another massive waterborne outbreak was reported in Milwaukee, WI. Contamination of the public water supply during March and April 1993 resulted in approximately 300,000 infections. Infection during this outbreak appeared to be more severe than in previous reports of large case series. The risk of secondary infection in a household was low (5%) when the index case involved an adult. Also, the recurrence of watery diarrhea after apparent recovery was a frequent occurrence among visitors with laboratory-confirmed cryptosporidiosis (39%) and among visitors and residents with clinical infection (21%).

This increase in the number of reported waterborne disease outbreaks associated with *Cryptosporidium* spp. can be attributed to improved techniques for oocyst recovery and identification. The use of high-volume filters, immunofluorescence, and molecular detection methods has resulted in the demonstration of oocysts in surface and drinking waters and in sewage effluents from many geographic regions (21, 22, 24–27).

TABLE 23.15 Possible surveillance approaches for the establishment of baseline data on the occurrence of cryptosporidiosis

Surveillance approach	Comments
Make cryptosporidiosis reportable to CDC	Applies to each state or city; provides legal authority for collecting information
Monitor sales of antidiarrheal medications	Local pharmacy computerized databases (provide historical as well as current diarrheal illness information)
Monitor logs maintained by health maintenance organizations and hospitals (complaints of diarrheal illness)	May be particularly useful if keyed to ZIP codes, particularly in case of waterborne transmission
Monitor incidence of diarrheal illness in nursing homes	Can monitor differences between use of municipal and bottled water; could also serve to establish baseline data
Monitor laboratory data for *Cryptosporidium* spp.	More likely to provide information on AIDS (or other immunocompromised) patients; may not be best way to obtain information on general public (excessive cost)
Monitor tap water in selected cities or locations	Intensive surveillance might be recommended in approximately 10 cities known to have *Cryptosporidium* spp. in their finished water, again to establish some baseline data
Establish methods of rapid reporting and communication among relevant agencies	Education of physicians, public, etc., is vital to the understanding of epidemiology of cryptosporidiosis

Water Testing. The immunofluorescence method can be used to examine large volumes of water, is relatively specific for *Giardia* and *Cryptosporidium* spp., and permits examination of the specimen by epifluorescence and phase-contrast or differential interference contrast microscopy. However, disadvantages of the method include low recovery percentages (5 to 25%), long processing times (1–2 days to 1–2 weeks), the need for highly trained personnel, the high cost per sample (approximately $300), the inability to identify viable or virulent organisms, and cross-reactivity with non-human *Cryptosporidium* organisms. Unfortunately, the more the specimen or water sample is manipulated during processing, the more likely it is that organisms will be lost in the process, thus accounting for the low recovery percentages.

Incorporation of the use of fluorogenic vital dyes to determine oocyst viability appears to offer some improvements; however, the positive relationship between vital-dye reactions and excystation may not always correlate directly with viability or infectivity. A number of other methods have been used in an effort to improve organism recovery and to determine the viability and infectivity of the oocysts recovered. These include the use of a fluorescence *in situ* hybridization technique for labeling of *Cryptosporidium* oocysts in water samples, species- and strain-specific typing of parasites in clinical and environmental samples, use of PCR for the detection of *Cryptosporidium* oocysts in municipal water samples, use of cell culture to determine the infectivity of *Cryptosporidium* oocysts, immunomagnetic separation coupled with immunofluorescence microscopy for oocyst detection, a combination of immunomagnetic separation and integrated cell culture-PCR, and use of β-tubulin mRNA as a marker of *Cryptosporidium* oocyst viability.

Data indicate that viable *Cryptosporidium* oocysts exposed to chlorine or other oxidizing conditions may lack identifying epitopes. This finding may be important for the water industry, where naturally occurring oxidizing conditions or sanitizing treatments could produce viable oocysts that are undetectable by standard protocols. Detailed knowledge of the structure and stability of the *Cryptosporidium* oocyst wall may ultimately contribute to the development of water treatment options that will reduce the viability of the intact oocyst and thus the waterborne dissemination of the parasite.

Cyclospora cayetanensis

The first published report of *Cyclospora* infection in humans can probably be dated to 1979 when Ashford described coccidian organisms causing diarrhea in two children and a woman in Papua New Guinea and concluded that they could be *Cystoisospora*. In 1986, four travelers returning from Mexico and Haiti had flu-like symptoms and

TABLE 23.16 Potential sources for *Cryptosporidium* oocysts related to accidental human ingestion of contaminated food and/or water

Transmission agent	Comments
Eastern oysters (*Crassostrea virginica*)	Oysters collected from Maryland tributaries of the Chesapeake Bay contain infectious oocysts; 7 sites in Chesapeake Bay area are positive for infected oysters
Atlantic coast commercial shellfish	*Cryptosporidium* spp. found in commercial shellfish from 64.9% of sites examined along the Atlantic coast, using either microscopy or molecular testing
Clams (Italy, seawater)	Oocysts detected in 23 of 32 pools of clams
Clams (California, freshwater)	*C. parvum* DNA in clams from 3 different riverine ecosystems confirmed with PCR and DNA sequence analysis
Clams (*Tapes decussates*, Spain)	Oocysts found in siphons, gills, stomach, digestive diverticula, intestine; oocysts found in branchial mucus and in interfilamentary spaces suggest repeated filtrations and the possibility of infective oocysts
Bent mussels (*Ischadium recurvum*)	Mussels may be useful as biological indicator of water contamination with *Cryptosporidium* oocysts; found in same areas indicated above for oysters
Mussels (*Mytilus galloprovincialis*)	Oocysts survive in seawater for at least 1 year; filtered out by benthic mussels, retaining infectivity up to 14 days
Mussels (*Perna viridis*); Thailand	*Cryptosporidium* spp. oocysts indigenous in mussels from coast of Thailand (food source)
Canada geese (migrating birds)	Infectious oocysts disseminated by migrating waterfowl
House flies (*Musca domestica*)	Adult and larval stages of house flies can mechanically carry oocysts in their digestive tracts and deposit them on external surfaces
Filth flies (*Calliphoridae*, *Sarcophagidae*, *Muscidae*)	Wild-caught flies (>90%) harbored *Cryptosporidium* oocysts; oocysts carried internally and externally—potential for mechanical transmission of infectious oocysts
Filth flies (*Muscidae*, *Calliphoridae*, *Lauxaniidae*, *Anthomyiidae*)	80% of all *Cryptosporidium* oocysts viable; >69% of *Giardia lamblia* cysts viable; potential for mechanical transmission of infectious oocysts high
Dung beetles (*Anoplotrupes stercorosus*, *Aphodius rufus*, *Onthophagus fracticornis*)	Although many oocysts pass safely through mouthparts and gastrointestinal tracts of the beetles, most are destroyed; however, coprophagous insects are considered important as agents of both control and dissemination
Free-living soil nematode (*Caenorhabditis elegans*)	*C. elegans* can ingest and excrete oocysts; the role of free-living nematodes in contamination requires further study

it was suggested that the cause was a new pathogen. In subsequent reports, *Cyclospora* was described as a coccidian-like body (CLB), cyanobacterium-like body, blue-green alga, or large *Cryptosporidium*. In 1989, 53 cases of CLB infection in Peru were attributed to an unidentified flagellate. In 1989, similar structures were seen in the stool samples of an AIDS patient in Chicago with chronic diarrhea and with no history of recent travel. Then, in the early 1990s, blue-green algae were described as the causative agent of severe diarrhea in eight international travelers who had been to Mexico, South America, India, or Southeast Asia and also in expatriates living in Nepal. It was not until the early 1990s that these organisms were characterized as a new coccidian species capable of causing human infection (40).

In recent years, human cyclosporiasis has emerged as an important infection, with a number of outbreaks being reported in the United States and Canada. Prior to 1995, only one outbreak of cyclosporiasis had been reported in the United States; however, from May through August 1996, more than 1,400 cases were reported from 20 states, Washington, DC, and two Canadian provinces (5, 41, 42). Understanding of the biology and epidemiology of *C. cayetanensis* is limited and has been complicated by a lack of information about the parasite's origins, possible animal reservoir hosts, and relationship to other coccidia (40–43).

There have been a number of outbreaks of diarrhea associated with *C. cayetanensis*; the distribution is worldwide (United States, Caribbean, Central and South America, Southeast Asia, Eastern Europe, Australia, Nepal). These organisms are acid-fast variable and have been found in the feces of immunocompetent travelers to developing countries, immunocompetent subjects with no travel history, and patients with AIDS. Patients reported symptoms of a "flu-like" illness with nausea, vomiting, anorexia, weight loss, and explosive diarrhea lasting 1 to 3 weeks. Cumulative evidence suggests that outbreaks in the United States and Canada during the spring months of 1996 and 1997 were related to the importation and ingestion of Guatemalan raspberries. It is quite likely that the outbreak reported from Florida in 1995 was also attributable to contaminated food. The cases reported in all three outbreaks probably represented only a small fraction of those that occurred. Rapid transport of fresh fruits and produce from developing countries increases the chance that endemic parasites from other regions may come into contact with consumers from industrialized nations; *Cyclospora* represents an excellent example of this type of transmission.

Life Cycle and Morphology

The oocysts that had previously been recovered from human stool specimens were immature, so the structure of the mature oocyst was not seen in those specimens. It takes approximately 7 to 15 days for oocyst maturation, so the mature stage may not have been seen in human specimens. Evidence obtained from excystation experiments indicates that the oocyst contains two sporocysts, each containing two sporozoites, a pattern which places these organisms in the coccidian genus *Cyclospora*. EM confirmed the presence of characteristic organelles for coccidian organisms of the phylum Apicomplexa. The name *Cyclospora cayetanensis* sp. nov. was proposed for this newly described disease-producing coccidian organism from humans. The species name comes from the university where it was initially studied (Universidad Peruana Cayetano Heredia).

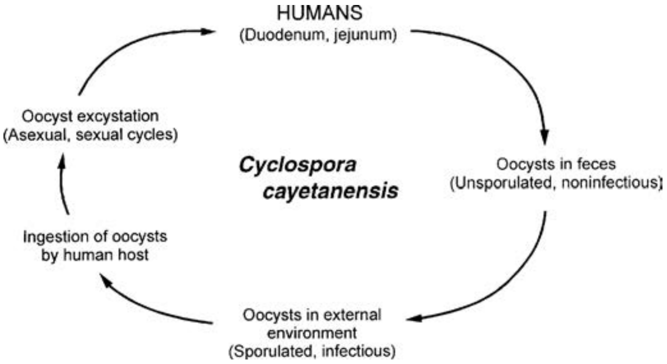
Two types of meronts and sexual stages have been seen in jejunal enterocytes in biopsy specimens from infected patients. These findings confirm that the entire life cycle can be completed within a single host (Fig. 23.9). Unsporulated oocysts are passed in the stool, and sporulation occurs within approximately 7 to 13 days. Complete sporulation produces two sporocysts that rupture to reveal two crescent-shaped sporozoites measuring 1.2 by 9.0 µm.

The transmission of *Cyclospora* is thought to be fecal-oral, although direct person-to-person transmission has not been well documented and may not be a factor, since sporulation takes a number of days. Outbreaks linked to contaminated water and various types of fresh produce (raspberries, basil, baby lettuce leaves, and snow peas) have been reported, and this organism is highly resistant to disinfectants commonly used in the food industry (Table 23.17, Fig. 23.10). The adhesins responsible for the strong attachment of *Cyclospora* to produce are unknown; however, it seems to be stronger than that of *Cryptosporidium* or *Giardia*. Also, information on potential reservoir hosts has yet to be defined; however, it appears that in some areas humans are the only host.

Clinical Disease

The clinical presentation varies throughout areas of endemicity, where asymptomatic infections are more frequent. Children tend to have more severe clinical symptoms. The infections cause fewer symptoms as children get older and as the duration of the infection decreases. As in young children, the elderly may also present with a more severe illness. In areas where *Cyclospora* is not endemic, infections almost always cause symptoms, and there may be serious sequelae; however, fatalities tend to be rare.

Figure 23.9 *Cyclospora cayetanensis* life cycle. doi:10.1128/9781555819002.ch23.f9



The incubation period is approximately 2 to 11 days after exposure. There is generally 1 day of malaise and low-grade fever, with rapid onset of diarrhea from 5 to 15 times per day. There may also be fatigue, anorexia, vomiting, myalgia, and weight loss with remission of self-limiting diarrhea in 3 to 4 days, followed by relapses lasting from 4 to 7 weeks.

In patients with AIDS, symptoms may persist for as long as 12 weeks; biliary disease has also been detected in this group. Diarrhea alternating with constipation has also been reported; this is not uncommon in a number of protozoal gastrointestinal infections. Clinical clues include unexplained prolonged diarrheal illness during the summer in any patient and in persons returning from tropical areas. The majority of infected individuals had intermittent diarrhea for 2 to 3 weeks, and many complained of intense fatigue, as well as anorexia and myalgia, during the illness. The clinical presentation of patients infected with this organism is similar to that of patients infected with *Cryptosporidium*.

Developmental stages of *C. cayetanensis* usually occur within epithelial cells of the jejunum and lower portion of the duodenum. *Cyclospora* infection reveals characteristics of a small bowel pathogen, including upper gastrointestinal symptoms, malabsorption of D-xylose, weight loss, and moderate to marked erythema of the distal duodenum. Alterations of the epithelial tissue include vacuolization at the tips of the villi of the surface epithelium, loss of the brush border, and alteration of cells from a columnar to cuboid shape. Histopathology in small bowel biopsy specimens reveals acute and chronic inflammation, partial villous atrophy, and crypt hyperplasia (Fig. 23.11).

Diagnosis

Electron Microscopy. When examined in formalin-preserved human stool specimens and by EM, these organisms exhibited characteristics of a cyanobacterium. The organisms stained orange with safranin and were modified acid-fast variable, with some organisms staining deep red with a mottled appearance but no internal organization (44). Preliminary EM revealed internal structures that resembled those of the cyanobacteria.

Autofluorescence. The oocysts autofluoresce strong green (450 to 490 DM excitation filter) or intense blue (365 DM excitation filter) under UV epifluorescence. It is strongly recommended that during concentration (formalin-ethyl

TABLE 23.17 Examples of outbreaks of *Cyclospora cayetanensis* infection, United States and Canada[a]

Outbreak	Implicated cause	Comments
July 1990 (11 cases), Chicago hospital	Tap water from the physicians' dormitory was probably the source of the outbreak	This is the first reported outbreak of diarrhea associated with *Cyclospora* in the United States.
1995 (14 cases), Florida	Fresh raspberries and strawberries	Given the cumulative evidence and the occurrence in 1996 and 1997 of outbreaks in North America associated with consumption of Guatemalan raspberries, food-borne transmission of *Cyclospora* was likely in 1995 in Florida as well.
23 May 1996 (38 cases), South Carolina	Fresh raspberries	Imported from Guatemala
26 June 1996 (48–54 possible cases), Virginia	Basil-pesto pasta salad	No raspberries or mesclun lettuce was served at the luncheon.
June and July 1996 (185 possible cases), 25 clusters in northern Virginia, Washington, DC, and Baltimore, MD	Food containing fresh basil	All basil was associated with the same company.
1996 (180 cases), Florida	Fresh raspberries	Imported from Guatemala
1996 (1,465 cases), 20 states	Fresh berries, berries with or without fresh raspberries	As few as five Guatemalan farms could have accounted for the 25 events; the raspberries could be traced to a single exporter per event.
April and May 1997 (80 cases), California, Florida, Nevada, New York, and Texas	Fresh raspberries, mesclun (baby lettuce leaves, known as spring mix, field greens, or baby greens)	These 80 cases a part of the next listing for 1997 of a total of 1,450 cases
1997 (1,450 cases), United States and Canada	Fresh raspberries, mesclun	Most cases were linked to ingestion of raspberries from Guatemala; exportation of fresh raspberries from Guatemala was voluntarily suspended at the end of May 1997.
1998 (94 cases), Boston, MA	Dessert containing raspberries, strawberries, blackberries, and blueberries	No specific commentary on source of fresh berries, but probably imported
July 1999 (62 cases), Missouri	Fresh basil	Chicken pasta salad and tomato basil salad; grown either in Mexico or United States
June 2000 (54 cases)	Fresh raspberries	Wedding cake, which had a cream filling that included raspberries imported from Guatemala
2001 (17 cases), British Columbia	Fresh Thai basil	Importation was traced from the United States.
June and July 2004 (96 cases), Pennsylvania	Pasta salad containing raw snow peas	Snow peas imported from Guatemala
April 2005 (592 cases), Florida	Fresh basil	Linked to a number of restaurants
April 2009 (160 cases), multiple countries	Unknown	Cruise ship
2012 (123 cases)	Possible cilantro	Restricted shipments from Puebla, Mexico (FDA, 2015)
August 2013 (631 cases), 22 states	Prepackaged salad mix	Lettuce grown in Mexico, restaurant salads, largest U.S. outbreak since 1997; 83% of cases from Iowa, Nebraska, Texas

[a] During this time frame, outbreaks were also being documented from many areas throughout the world (Canada, Germany, Mexico, Indonesia, Colombia, Peru, Turkey, Nigeria, India, China, Sweden).

acetate) of stool specimens, the centrifugation be carried out for 10 min at 500 × *g*.

Modified Acid-Fast and Safranin Stains. In clean wet mounts, the organisms are seen as nonrefractile spheres and are acid-fast variable with the modified acid-fast stain; those that are unstained appear as glassy, wrinkled spheres (Fig. 23.12 to 23.14). Modified acid-fast stains stain the oocysts light pink to deep red, and some will contain granules or have a bubbly appearance. It is very important to be aware of these organisms when the modified acid-fast stain is used, because *Cryptosporidium* spp. and other similar but larger structures (approximately twice the size of *Cryptosporidium* oocysts) are seen in the stained smear. It is important for laboratories to measure all acid-fast oocysts, particularly if they appear to be somewhat larger than those of *Cryptosporidium*. Although modified acid-fast stains are the most commonly used stains to identify the oocysts in stool, a safranin-based stain that tends to uniformly stain *Cyclospora* oocysts is also recommended (44).

Flow Cytometry. Another diagnostic option involves the use of flow cytometry. This approach appears to be a useful alternative to microscopy, particularly for screening large

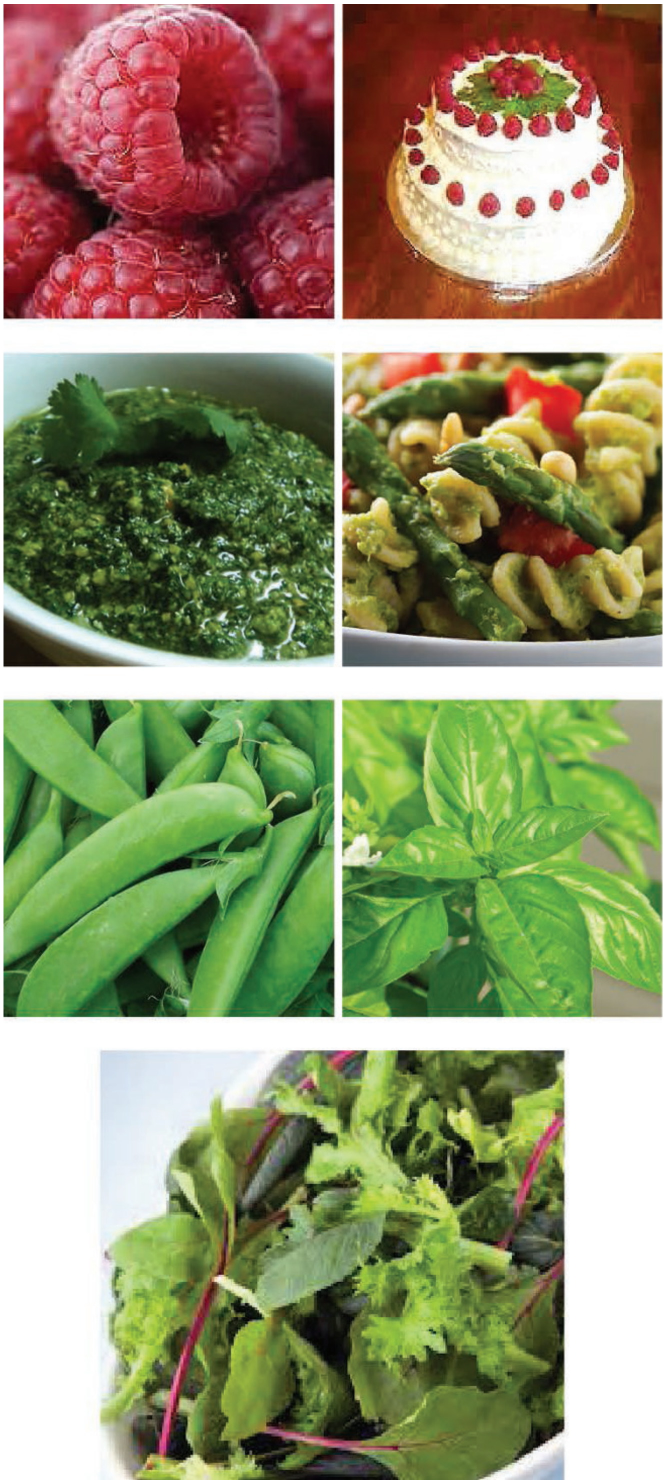


Figure 23.10 Foods associated with *Cyclospora cayetanensis* contamination and transmission. (Top) Raspberries, raspberry cake; (row 2) pesto, pesto salad; (row 3) snow peas, basil; (bottom) mesclun (baby lettuce leaves for salad).
doi:10.1128/9781555819002.ch23.f10

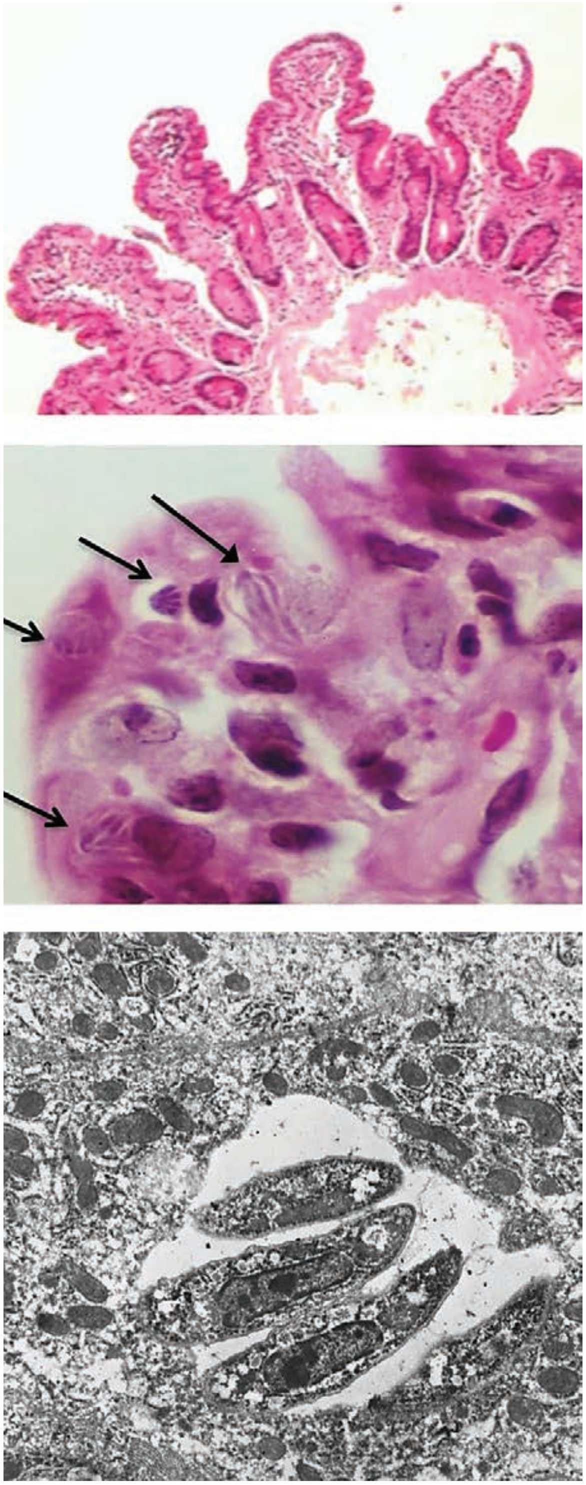


Figure 23.11 *Cyclospora cayetanensis*. (Top) Histopathological changes of the intestinal epithelia in an individual with cyclosporiasis (note some blunting of villi). (Middle) Multiple parasitic vacuoles containing meronts type 1 and 2 (arrows). (Bottom) Transmission EM, parasitic vacuole containing fully differentiated meronts. (Courtesy of Ynes Ortega.)
doi:10.1128/9781555819002.ch23.f11

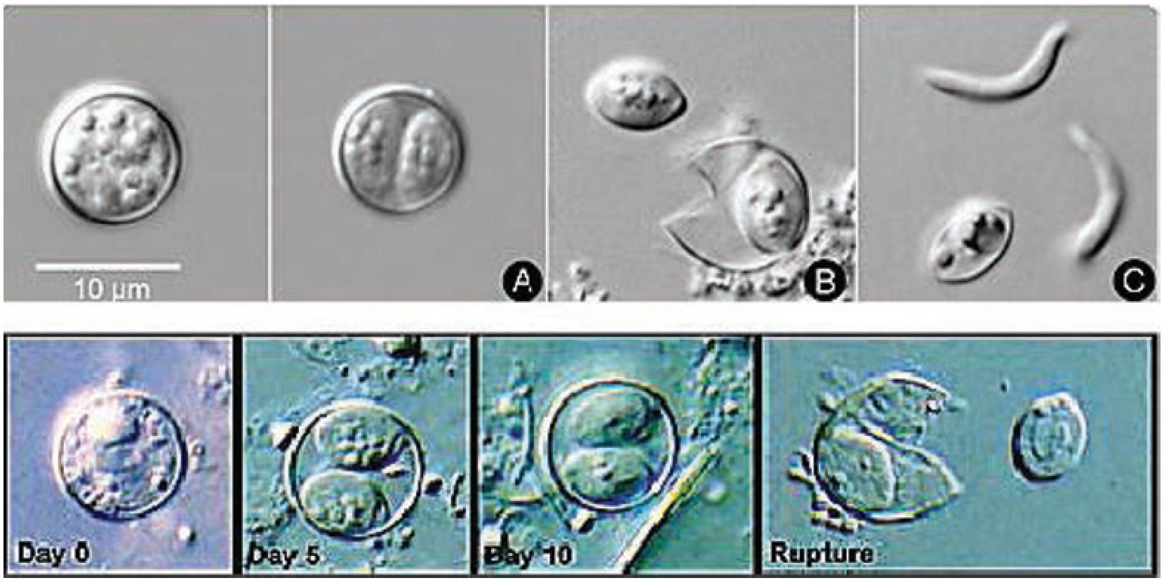


Figure 23.12 *Cyclospora cayetanensis* oocysts. (Upper) (far left) Unsporulated oocyst with undifferentiated cytoplasm; (A) sporulating oocyst that contains two immature sporocysts; (B) oocyst that was mechanically ruptured with one of its two sporocysts released; (C) one free sporocyst is seen, as well as two free sporozoites, the infective stage of the organism. (Lower) Sporulation of *Cyclospora* oocysts; Day 0, oocyst passed in fresh stool; Days 5 and 10, sporulated oocysts containing two sporocysts; Rupture, ruptured oocyst, one sporocyst outside, one remaining inside the oocyst wall. (Courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch23.f12

numbers of clinical specimens for *Cyclospora* oocysts in an outbreak situation (45).

Molecular Testing. *Cyclospora* DNA amplification by PCR has been used for diagnosis. Conventional PCR and nested PCR targeting small subunit rRNA for *Cyclospora* have a sensitivity of 62%. False positivity with some *Eimeria* species has been noted (40). The amplified product of about 300 bases cannot differentiate *Cyclospora* from *Eimeria* species. Digestion of the amplified products using the restriction enzyme MnlI (PCR-restriction fragment length polymorphism [PCR-RFLP]) followed by gel electrophoresis results in patterns that can discriminate these two genera. Recently, a 70-kDa heat shock protein (HSP70) gene-based nested PCR protocol for detection of *C. cayetanensis* has been developed (46). The BioFire Film Array Gastrointestinal Panel for bacterial, viral, and pathogenic pathogens is now available and includes *Cyclospora* (Table 23.4).

Antibody Testing. Serological assays to determine human exposure to *Cyclospora* are not yet available. Westerm blot can differentiate between acute and convalescent infection; however, this test is still in the research stages.

KEY POINTS—LABORATORY DIAGNOSIS

Cyclospora cayetanensis

1. *Cyclospora* oocysts may appear to be larger than normal *Cryptosporidium* oocysts; be sure to measure these organisms to confirm their size.
2. Fecal specimens should be concentrated prior to staining; centrifugation for 10 min at 500 × *g* is recommended.
3. On wet smears, the oocysts appear as nonrefractile spheres and, depending on the filters used, autofluoresce green to blue with epifluorescence. Autofluorescence may be 2+ to 4+.
4. On modified acid-stained smears, the oocysts stain from light pink to deep red, and some contain granules or have a bubbly appearance. Those that do not stain may appear wrinkled. A strong decolorizer should not be used; a 1% sulfuric acid solution is recommended and also works well for modified acid-fast stains for *Cryptosporidium* spp. and/or *Cystoisospora belli*. The original 3 to 5% sulfuric acid is usually too strong for *Cyclospora* and removes too much color. Even with the 1% acid decolorizer, some oocysts may appear clear or very pale.
5. If the oocysts are seen in a regular trichrome-stained smear of stool, they may appear as clear, round, some-

(continued)

KEY POINTS—LABORATORY DIAGNOSIS

***Cyclospora cayetanensis* (continued)**

what wrinkled objects that are larger than *Cryptosporidium* spp. However, unless there are large numbers of these oocysts present, they look like "ghost cells" and will probably not be recognized as *Cyclospora* oocysts.

6. It is very important to measure these oocysts; *Cyclospora* oocysts measure 8 to 10 µm, and *Cryptosporidium* oocysts measure from 4 to 6 µm.
7. In the modified safranin technique, the oocysts uniformly stain a brilliant reddish orange if fecal smears are heated in a microwave oven during staining. The stained slide can also be examined by epifluorescence microscopy first, and suspect oocysts can be confirmed by bright-field microscopy.

Treatment

Apparently, patients do not respond to conventional antimicrobial therapies. Some patients have been treated symptomatically with antidiarrheal preparations and have obtained some relief; however, the disease appears to be self-limiting within a few weeks. Trimethroprim-sulfamethoxazole (TMP-SMX) is currently the drug of choice (adults: 760/800 mg given orally twice daily for 7 days; children: 5 mg of TMP/kg plus 25 mg of SMX/kg given orally twice daily for 7 days); relief of symptoms has been seen by 1 to 3 days posttreatment. Patients with AIDS may need higher doses and long-term maintenance treatment. However, symptoms recur within 1 to 3 months posttreatment in over 40% of patients. In a study with TMP-SMX in Nepal, shedding of oocysts and symptoms disappeared after 7 days of treatment (43).

Other antibiotics, such as azithromycin, norfloxacin, tinidazole, nalidixic acid, diloxanide fluorate, and quinacrine, have also been tested for *Cyclospora* treatment, all without success. Ciprofloxacin can be used as an alternative therapy in patients who are allergic to sulfa products; however, evidence of treatment failure with ciprofloxacin has also been seen.

Epidemiology and Prevention

Epidemiology. Epidemiological studies have shown that consumption of untreated water, lack of adequate sanitation, and the presence of animals in the household are associated with increased risk of *Cyclospora* infections. In industrialized nations, most people are susceptible to infections. However, susceptible populations in areas of endemicity are restricted to the very young and the very old. In developed nations, cyclosporiasis has been observed in tourists (0.6%) or expatriates visiting countries where the disease is endemic, in the immunocompromised, and in immunocompetent hosts with no foreign travel history. Published reports emphasize that individuals of all ages, including both those who are immunocompetent and those who are immunosuppressed, can become infected. Travelers to areas with endemic *Cyclospora* infection should be aware of the increased risk of infection during the late spring and summer. Preliminary data and extrapolation from what we currently know about cryptosporidiosis suggest that modes of transmission may be similar, particularly considering waterborne transmission (Table 23.11).

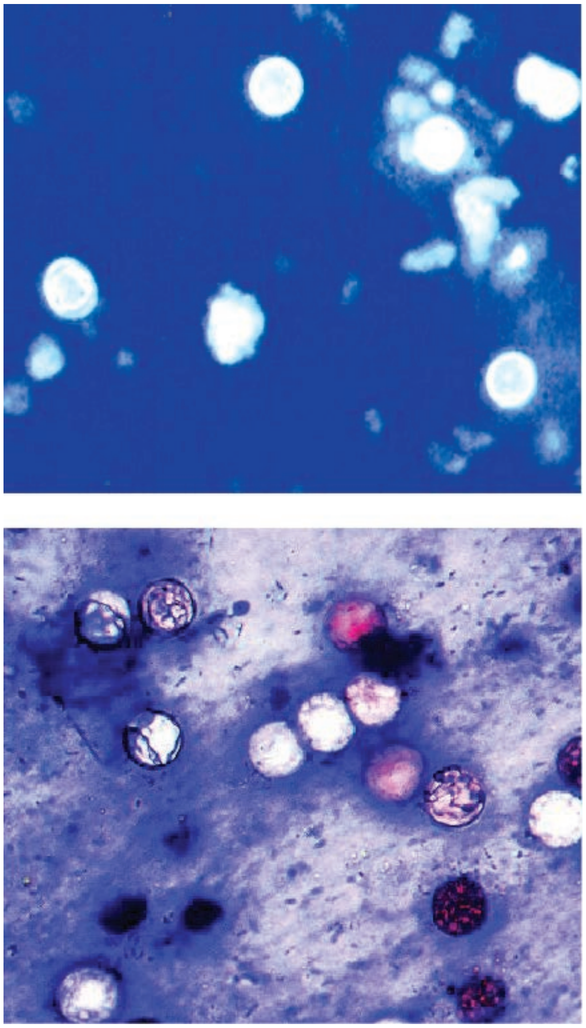


Figure 23.13 (Upper) *Cyclospora cayetanensis* autofluorescence using the same filter used for calcofluor white. (Lower) Modified acid-fast stain of *C. cayetanensis* (note that some oocysts stain quite dark, while some have not retained the stain; this is typical, and the organisms are described as "modified acid-fast variable"). The oocysts measure 8 to 10 µm. A 1% acid decolorizer is recommended—all coccidia will stain well using this approach. A stronger acid may decolorize *Cyclospora* too much. **Note** These images were photographed from concentrated, stained fecal material (500 × *g* for 10 min). doi:10.1128/9781555819002.ch23.f13

Oocyst detection methods for foods and environmental samples include phase-contrast, bright-field, and differen-

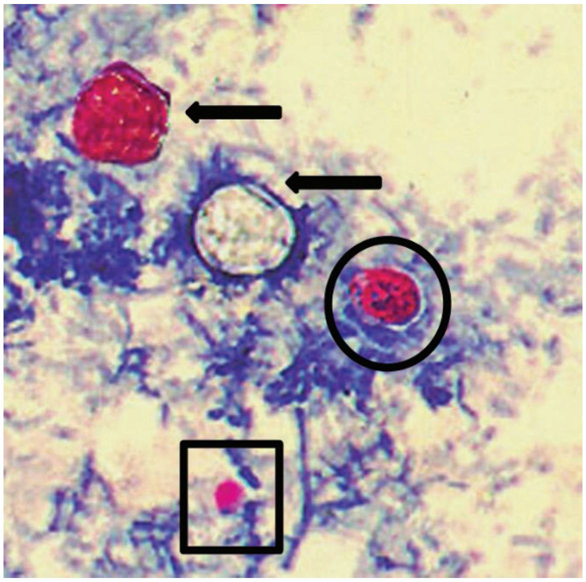


Figure 23.14 Modified acid-fast stain. Oocysts of *Cryptosporidium* (4–6 µm) (circle) and *Cyclospora cayetanensis* (8–10 µm) (arrows); one of the *Cyclospora* oocysts has not retained the stain. The smallest structure (square) is an artifact and measures approximately 1–2 µm.
doi:10.1128/9781555819002.ch23.f14

tial interference contrast microscopy. Epifluorescence microscopy has also been used. Other coccidian oocysts also autofluoresce and stain with acid-fast stain, so these procedures have to be used in combination with other diagnostic assays.

Molecular Epidemiology. Although current PCR protocols can detect *Cyclospora* in different samples, these methods cannot differentiate between sporulated and unsporulated oocysts, limiting their use in biological or epidemiological investigations. Therefore, there are no animal models or *in vitro* cultivation methods to determine the infectious potential of *Cyclospora*.

Prevention. Since this parasite, like *Cryptosporidium*, is not killed by routine chlorination, drinking water treated by halogenation may not be safe. Boiling is recommended. Hot drinks such as coffee or tea are recommended, rather than placing any reliance on iodine treatment of the water. Fresh fruits and vegetables should be thoroughly washed and/or peeled prior to eating. Although prophylaxis has not been adequately investigated and is not recommended, specific circumstances might warrant this approach (e.g., for HIV-positive individuals visiting these areas).

Improved agricultural practices would contribute to reducing the burden of parasite contamination at the farm level. These practices would involve the use of properly treated irrigation water and the use of pathogen-free water for washing produce.

As our understanding of the epidemiology of cyclosporiasis expands, it is obvious that there may be numerous ways for the resistant oocysts to become disseminated. Some of these recent examples identified for *Cryptosporidium* spp. have generated enhanced interest in and concern about additional sources of infectious oocysts and the potential for accidental human consumption through contaminated food and/or water. These examples may be just as relevant for *Cyclospora*. Specific examples for *Cryptosporidium* can be seen in Table 23.16. Recovery of waterborne oocysts of *C. cayetanensis* in Asian freshwater clams (*Corbicula fluminea*) has been reported (42, 43). It has been proposed that shellfish concentrate oocysts from contaminated waters. Controlled laboratory studies with *Corbicula fluminea* showed that 48 to 100% of the clams retained *Cyclospora* oocysts for up to 13 days.

Cystoisospora (*Isospora*) *belli*

Cystoisospora belli was first described by Virchow in 1860 but not named until 1923. Molecular biology studies have demonstrated that *Isospora* spp., particularly those from primates and carnivores, are more closely related to the *Sarcocystiidae* than to the *Eimeriidae*. This has required the transfer of the species into the family *Sarcocystiidae* and the genus *Cystoisospora*. Although infections have been found in various parts of the world, certain tropical areas in the Western Hemisphere appear to contain some well-defined locations of endemicity. These organisms can infect both adults and children, and intestinal involvement and symptoms are generally transient unless the patient is immunocompromised. *C. belli* has also been implicated in traveler's diarrhea (47). However, unlike *C. cayetanensis*, large outbreaks of cystoisosporiasis have not been reported.

Although oocysts of *Isospora natalensis* have been reported in patients from South Africa, the validity of this species requires further study and documentation.

Life Cycle and Morphology

Schizogonic and sporogonic stages in the life cycle of *C. belli* have been found in human intestinal mucosal biopsy specimens. Development in the intestine usually occurs within the epithelial cells of the distal duodenum and proximal jejunum. Eventually, oocysts are passed in the stool; they are long and oval and measure 20 to 33 µm by 10 to 19 µm (Table 23.2). Usually the oocyst contains only one immature sporont, but two may be present (Fig. 23.15 and 23.16). Continued development occurs outside the body with the development of two mature sporocysts, each containing four sporozoites, which can be recovered from the fecal specimen (Fig. 23.17, 23.18, 23.19, 23.20). The sporulated oocyst is the infective stage that will excyst in the small intes-

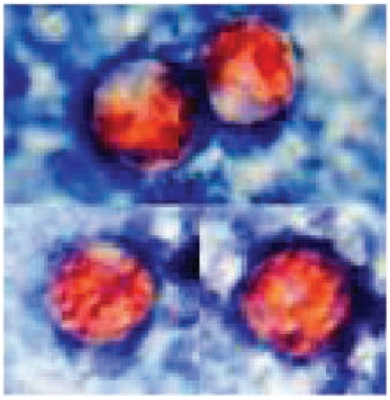
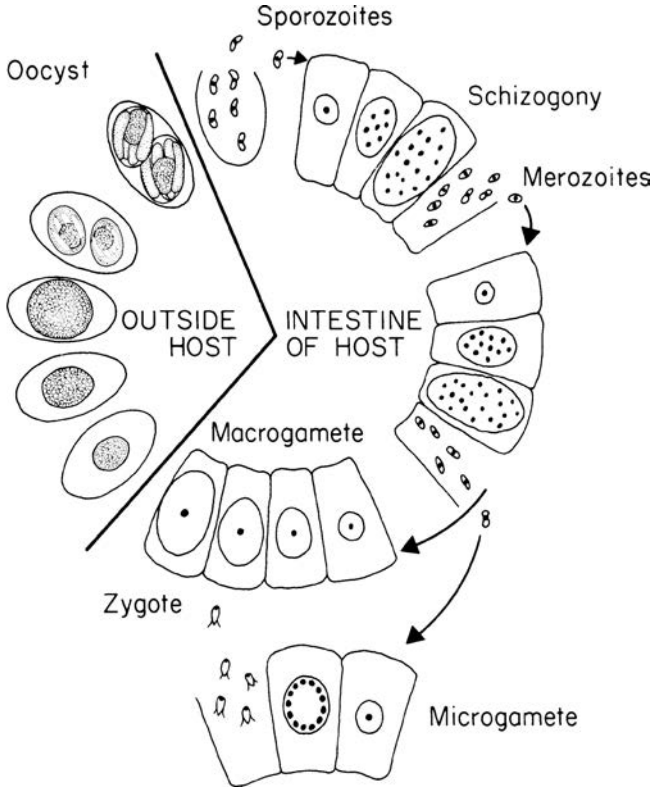


Figure 23.15 Hot safranin stain of *Cyclospora cayetanensis* oocysts (photographed at a high magnification). Note the oocysts are unsporulated. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch23.f15

tine, releasing the sporozoites, which penetrate the mucosal cells and initiate the life cycle. The life cycle stages, i.e., schizonts, merozoites, gametocytes, gametes, and oocysts, are structurally similar to those seen in the other coccidia.

The patent period is not known but may be as short as 15 days. Chronic infections develop in some patients, and oocysts can be shed for several months to years. In one particular case, an immunocompetent individual had symptoms for 26 years and *C. belli* was recovered in stool a number of times over a 10-year period. However, in such cases, one also has to take into account the possibility of reinfection.

Figure 23.16 Life cycle of *Cystoisospora belli*. (Illustration by Gwen Gloege.) doi:10.1128/9781555819002.ch23.f16



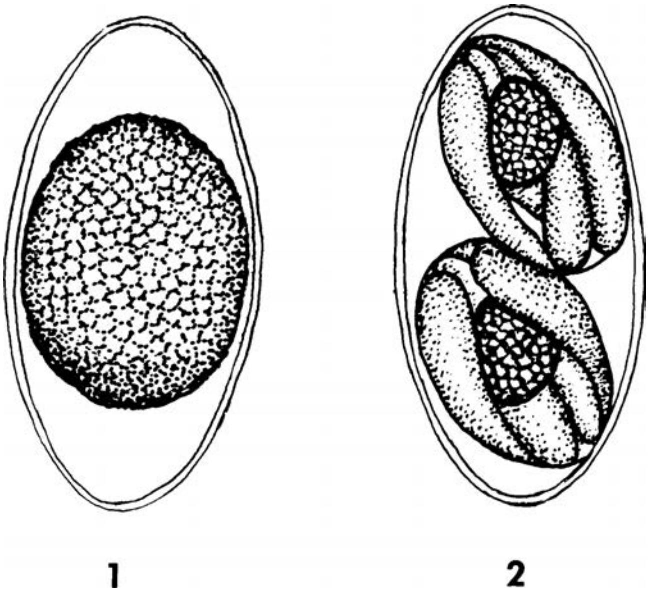
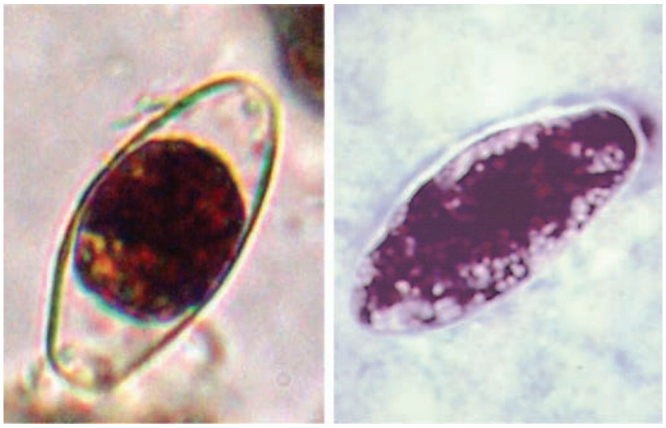


Figure 23.17 (1) Immature oocyst of *Cystoisospora belli*; (2) mature oocyst of *C. belli*. (Illustration by Nobuko Kitamura.) doi:10.1128/9781555819002.ch23.f17

Clinical Disease

Risk factors for symptomatic *C. belli* infection may include a variety of conditions. In addition to immunocompromised states that include AIDS, adult T-cell leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, thy-

Figure 23.18 (Left) Immature oocyst of *Cystoisospora belli*, wet preparation. (Right) Immature oocyst of *C. belli*, modified acid-fast stain (note that the entire oocyst has stained). doi:10.1128/9781555819002.ch23.f18



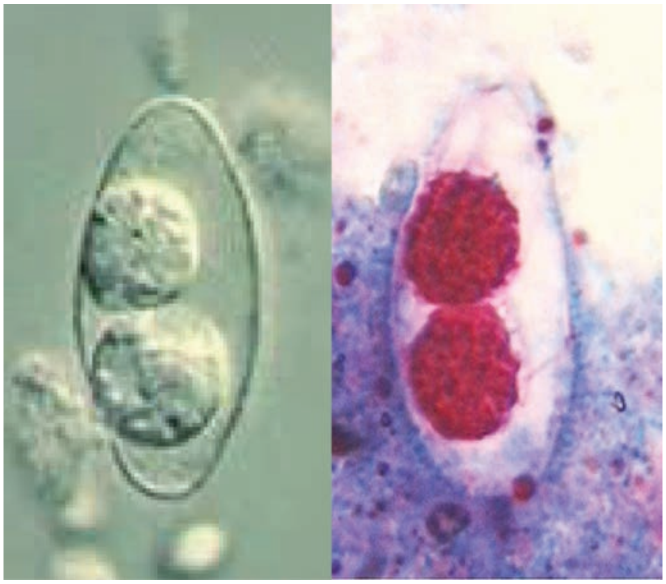


Figure 23.19 (Left) Mature oocyst of *Cystoisospora belli*, wet preparation. (Right) Mature oocyst of *C. belli*, modified acid-fast stain. doi:10.1128/9781555819002.ch23.f19

moma, transplantation, and corticosteroid-treated patients, immunocompetent conditions can also be the risk factors for symptomatic cystoisosporiasis. These factors include poor hygiene, malnutrition, chronic alcoholism, and debilitating infectious diseases.

Figure 23.20 *Cystoisospora belli*. (Upper) Immature oocyst photographed by fluorescence microscopy; (lower) mature oocyst photographed by fluorescence microscopy (courtesy of the CDC Public Health Image Library).
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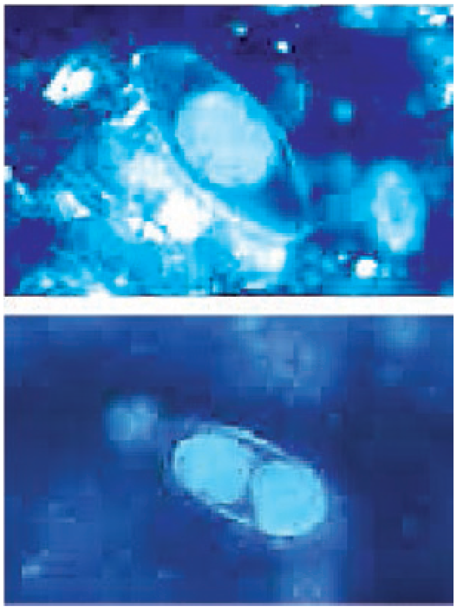

Clinical symptoms include diarrhea, which may last for long periods (months to years), weight loss, abdominal colic, and fever; diarrhea is the main symptom. Bowel movements (usually 6 to 10 per day) are watery to soft, foamy, and offensive smelling, suggesting a malabsorption process. Eosinophilia is found in many patients, recurrences are quite common, and the disease is more severe in infants and young children. Charcot-Leyden crystals derived from eosinophils have also been found in the stools of patients with *C. belli* infection. The diarrhea and other symptoms may continue in compromised patients, even those on immunosuppressive therapy, when the regimen of therapy is discontinued. This infection has been found in homosexual men, all of whom were immunosuppressed and had several months of diarrhea.

Immunosuppressed Patients. Patients who are immunosuppressed, particularly those with AIDS, often present with profuse diarrhea associated with weakness, anorexia, and weight loss. In one patient with a well-documented infection of long standing, a series of biopsies showed a markedly abnormal mucosa with short villi, hypertrophied crypts, and infiltration of the lamina propria with eosinophils, neutrophils, and round cells. It has been recommended that physicians consider *C. belli* in AIDS patients with diarrhea who have immigrated from or traveled to Latin America, are Hispanics born in the United States, are young adults, or have not received prophylaxis with TMP-SMX for *Pneumocystis jirovecii*. It has also been recommended that AIDS patients traveling to Latin America and other developing countries be advised of the waterborne and foodborne transmission of *C. belli*, and they may want to consider chemoprophylaxis.

Chronic severe watery diarrhea due to *C. belli* has also been reported in other immunodeficiency states. These include HIV-negative renal transplant recipients, human T-cell leukemia virus 1-associated T-cell lymphoma, acute lymphoblastic leukemia, Hodgkin's lymphoma, and thymoma.

Extraintestinal Infections. Extraintestinal infections in AIDS patients have been reported. One patient was a 38-year-old man who presented with progressive dyspnea and fever; he also complained of dysphagia, nausea, vomiting, and brown watery diarrhea (eight or nine episodes daily) and had lost 20 lb in 2 months. He was diagnosed with *P. jirovecii* pneumonia and oropharyngeal candidiasis and was treated with TMP-SMX and pentamidine. His condition improved, and he was discharged; however, he was readmitted complaining of nausea, vomiting, and diarrhea; he was diagnosed with giardiasis and treated. Five months later he was diagnosed as having *C. belli* and *Entamoeba histolytica* infection and was treated. Three months later he presented with dyspnea, fever, diarrhea, and generalized wasting; he was diagnosed with cytomegalovirus pneumonia and died 2 weeks later. At autopsy, microscopic findings associated with *C. belli* infection were seen in the lymph nodes and walls of both the small and large intestines. Intracellular zoites were seen in the cytoplasm of histiocytes. Each organism was surrounded by a thick eosinophilic cyst wall in routine histologic preparations with hematoxylin and eosin, and the cyst wall was periodic acid-Schiff (PAS) positive. Examination of the intestinal tissues revealed intraepithelial asexual and sexual stages of *C. belli*, as well as some merozoites that appeared to be in cells of the lamina propria.

The second case involved a 30-year-old woman who was living in France but was originally from Burkina Faso. Initially she was symptomatic with fever, diarrhea, and weight loss. She was diagnosed with esophageal candidiasis and *C. belli* infection. She was treated with TMP-SMX and given maintenance therapy but suffered episodes of recurrent infection over the next 3 years. Examination of samples collected at autopsy revealed stages of *C. belli* in the intestine, mesenteric and mediastinal lymph nodes, liver, and spleen. The extraintestinal stages were always observed as single organisms that did not stain well with acid-fast stains. Massive infection with plasmacytosis and some eosinophils, but no granulomatous reaction, was observed.

In a third AIDS patient who presented with watery, nonbloody diarrhea and fever, examination of small intestine biopsy specimens revealed merozoites in the intestinal lumen, lamina propria, and lymphatic channels. Finding the merozoites within the lymphatic channels documents a means of dissemination to lymph nodes and other tissues. Additional studies of extraintestinal tissue cysts have identified early tissue cysts that lack a developed cyst wall, demonstrated that more than one tissue cyst can occupy a host cell, described the distribution of micronemes and the shedding of zoite membranes, and identified tubular structures in the inner tissue cyst wall and inner compartment.

In another AIDS patient who was sporadically treated for HIV, tuberculosis, and *C. belli* diarrhea over a period of years, autopsy findings confirmed the presence of macrogametocytes and microgametocytes within the epithelium of the gallbladder and unicellular cysts in the mesenteric lymph nodes, as well as in the esophagus, duodenum, ileum, colon, and appendix.

Diagnosis

HIV and intestinal parasitic infections are among the main health problems in developing countries. Coinfections of these diseases often enhance the progression of HIV to AIDS. Laboratory diagnosis and identification of opportunistic intestinal parasites should be emphasized for patients with lower CD4$^+$ T-cell counts, particularly those with ≤350 cells/mm^3 (48). Varying degrees of peripheral blood eosinophilia are often seen in patients with cystoisosporiasis. In patients with intestinal inflammatory diseases with diarrhea and peripheral blood eosinophilia, cystoisosporiasis should be included among the possible causative agents.

Routine Methods. Examination of a fecal specimen for the oocysts is recommended. However, wet preparation examination of fresh material either as the direct smear or as concentrated material is recommended rather than the permanent stained smear (Fig. 23.5; Table 23.3). The oocysts are very pale and transparent and can easily be overlooked. They can also be very difficult to see if the concentration sediment is from polyvinyl alcohol-preserved stool. The light level should be reduced, and additional contrast should be obtained with the microscope for optimal examination conditions (Fig. 23.16, 23.18, 23.19, and 23.20).

The oocysts are acid fast and can also be demonstrated by using auramine rhodamine stains. Organisms tentatively identified by using auramine rhodamine stains should be confirmed by wet smear examination or acid-fast stains, particularly if the stool contains other cells or excess artifact material (more normal stool consistency).

It is quite possible to have a positive biopsy specimen but not recover the oocysts in the stool because of the small numbers of organisms present. Stool microscopy cannot be used to rule out cystoisosporiasis, and even intestinal biopsies can provide false-negative results.

Histology. Based on multiple biopsies, the jejunal mucosa reveals a nodular appearance and villous atrophy with loss of villi. Histopathology reveals various *C. belli* life cycle stages, including trophozoites, schizonts, merozoites, and macro- and microgamonts (Fig. 23.21 and 23.22) (49). The mucosal tissues reveal villous atrophy, crypt hyperplasia, and diffuse infiltrations of eosinophils, lymphocytes, and plasma cells, which are consistent with cystoisosporiasis.

Molecular Testing. Although no test reagents are available commercially, a real-time quantitative PCR (qPCR) assay has been developed using melting curve analysis to detect, differentiate, and identify DNA from coccidian species of animal health, zoonotic, and food safety concern. The qPCR assay consistently detected DNA from as few as 10 *Toxoplasma gondii* oocysts, while demonstrating that *C. cayetanensis*, *C. parvum*, *Cryptosporidium muris*, *T. gondii*, *Eimeria bovis*, *Eimeria acervulina*, *Isospora suis*, and *Sarcocystis cruzi* could each be identified by unique melting curves and could be differentiated based on melting temperatures (50).

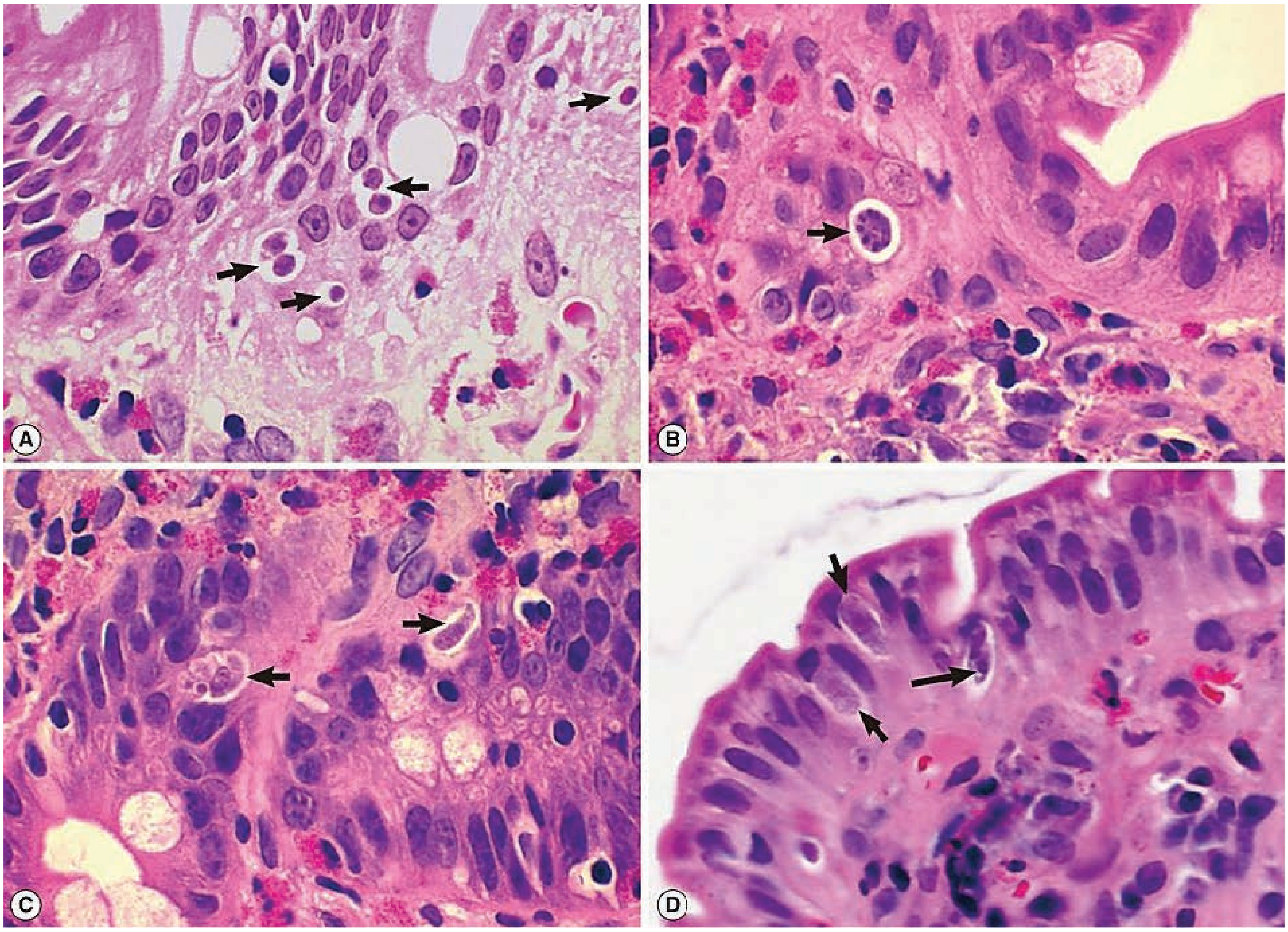

Figure 23.21 *Cystoisospora belli*. Sections of the upper jejunum showing various developmental (asexual and sexual) stages of *C. belli* (×1,000, hematoxylin and eosin stain). (A) Trophozoites (arrows), spherical in shape. (B) An immature schizont that has undergone nuclear division (arrow). Many eosinophils are infiltrated in the lamina propria. (C) A mature schizont with about six merozoites (left arrow) and a merozoite which has entered an enterocyte (right arrow). Eosinophil infiltrations are also seen in this figure. (D) Two merozoites in an enterocyte (long arrow), two macrogamonts (short arrows), and a developing trophozoite (lower left) are seen in the jejunal epithelial layer. (Courtesy of http://creativecommons.org/licenses/by-nc/3.0/.) doi:10.1128/9781555819002.ch23.f21

KEY POINTS—LABORATORY DIAGNOSIS

Cystoisospora belli

1. The oocysts are more easily recovered and identified when wet preparations are examined (direct smear, concentration smear). In a permanent stained trichrome or iron-hematoxylin smear, the oocysts may appear as debris due to heavy staining.
2. Oocysts recovered in a concentrated sediment from polyvinyl alcohol-preserved stool may be very difficult to detect (the oocyst wall is very difficult to see). However, examination of the sediment from stool preserved in other fixatives is satisfactory (vials containing no polyvinyl alcohol, newer single-vial collection vials).
3. A biopsy can be positive while no organisms are seen in the stool. This is not necessarily due to poor-quality laboratory work but may reflect normal findings, particularly when oocyst numbers are low.
4. Although these organisms can be stained with auramine-rhodamine stains, they should be confirmed by wet smears or modified acid-fast stains.

Treatment

Effective eradication of the parasites has been achieved with cotrimoxazole, TMP-SMX, pyrimethamine-sulfadiazine, primaquine phosphate-nitrofurantoin, and primaquine phosphate-chloroquine phosphate (33). Other drugs proven to be ineffective include dithiazanine, tetracy-

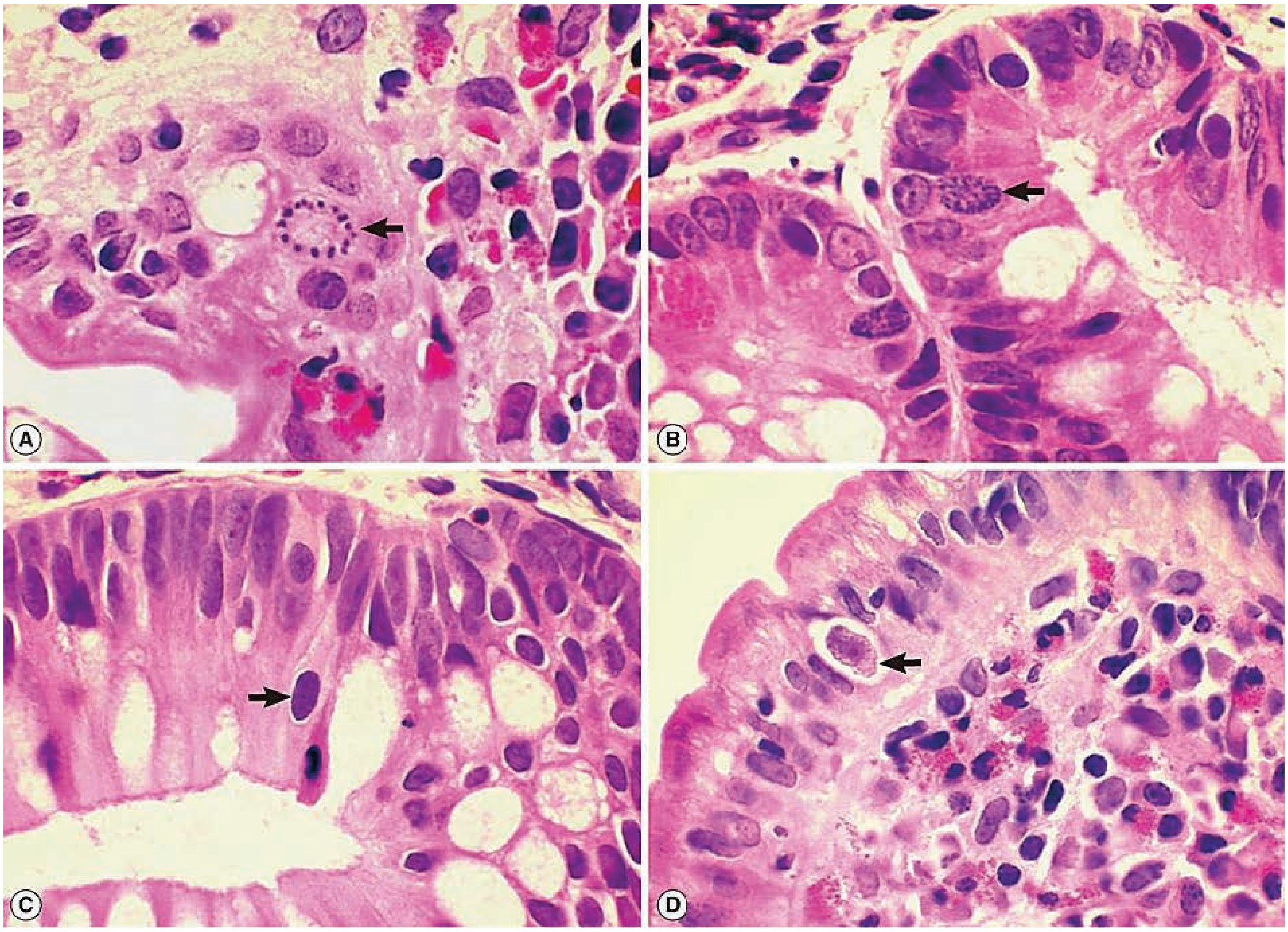

Figure 23.22 *Cystoisospora belli*. Sections of the upper jejunum showing various sexual stages of *Cystoisospora belli* (×1,000, hematoxylin and eosin stain). (A) A developing microgamont (arrow) with multiple nuclei that have migrated to the periphery. (B) A mature microgamont with multiple nuclei (arrow) in an enterocyte of the crypt layer. (C) A probable early macrogamont (arrow) in an enterocyte. (D) An old macrogamont or macrogamete (arrow) in a jejunal epithelial cell. (Courtesy of http://creativecommons.org/licenses/by-nc/3.0/.) doi:10.1128/9781555819002.ch23.f22

cline, metronidazole, phanquone, and quinacrine hydrochloride. The drug of choice is TMP-SMX, which is classified as an investigational drug for treatment of this infection. TMP (160 mg) plus SMX (800 mg) is given every 6 h for 10 days and then twice a day for 3 weeks. Pyrimethamine alone (50 to 75 mg daily) has cured infections in patients allergic to sulfonamides. However, chronic relapsing *C. belli* infections have been reported in AIDS patients despite treatment with TMP-SMX and immune reconstitution; in immunosuppressed patients with recurrent or persistent infection, therapy must be continued indefinitely. See chapter 36.

Epidemiology and Prevention

C. belli is thought to be the only species of *Cystoisospora* that infects humans, and no other reservoir hosts are recognized for this infection. Transmission is through ingestion of water or food contaminated with mature, sporulated oocysts. Sexual transmission by direct oral contact with the anus or perineum has also been postulated, although this mode of transmission is probably much less common. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist; oocysts usually mature within 48 h following stool evacuation and are then infectious. It has been speculated that diagnostic methods for laboratory examinations may tend to miss the organisms when they are present. Since transmission is via the infective oocysts, prevention centers on improved personal hygiene measures and sanitary conditions to eliminate possible fecal-oral transmission from contaminated food, water, and possibly environmental surfaces.

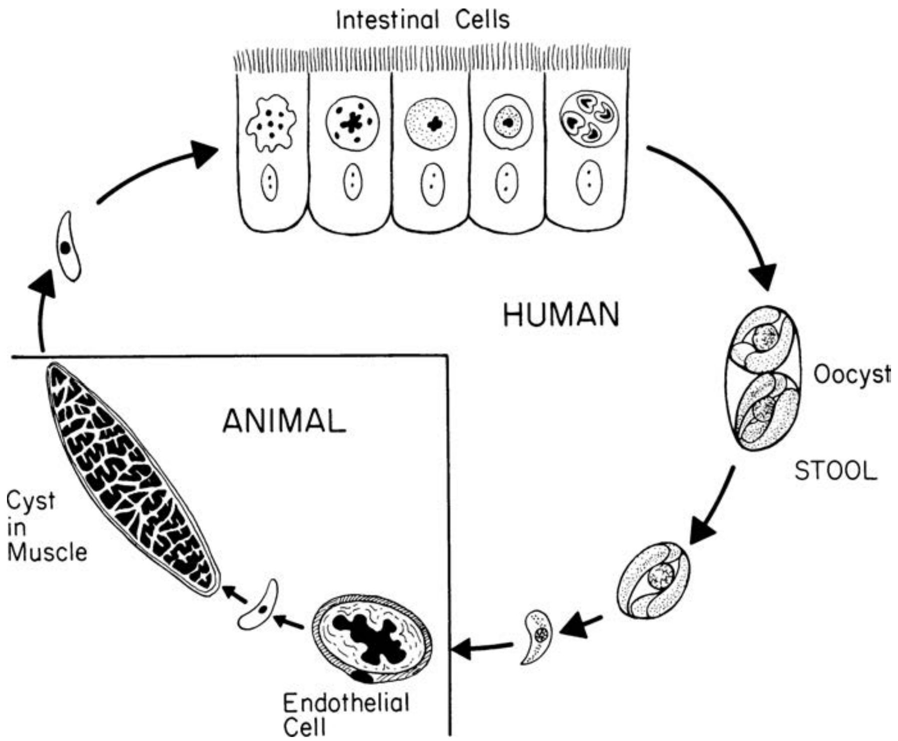

Figure 23.23 Life cycle of *Sarcocystis* spp. (Illustration by Gwen Gloege.)
doi:10.1128/9781555819002.ch23.f23

Sarcocystis spp.

The organism once known as *Isospora hominis* is now recognized as being a part of the life cycle of *Sarcocystis* spp. (Fig. 23.23). Two well-described species are *Sarcocystis hominis* (*bovihominis*) (cattle) and *S. suihominis* (pigs). When uncooked meat from these infected animals is ingested by humans, gamogony can occur within the intestinal cells, with the eventual production of the sporocysts in stool. In this case, humans who have ingested meat containing the mature sarcocysts serve as the definitive hosts (51, 52). There have been reports of fever, severe diarrhea, abdominal pain, and weight loss in immunocompromised hosts, although the number of patients with these symptoms has been quite small.

Life Cycle and Morphology

Species of *Sarcocystis* have an obligatory two-host life cycle. Intermediate hosts (including humans) such as herbivores and omnivores become infected through ingestion of sporocysts excreted in the feces of the definitive hosts such as carnivores and omnivores. The definitive hosts (including humans) become infected through ingestion of mature cysts found in muscles of the intermediate hosts. In some intermediate hosts, such as cattle and sheep, all adult animals may be infected (Table 23.18).

Sarcocystis hominis (bovihominis). Infection occurs when raw or poorly cooked infected meat is ingested by the human host. Known intermediate hosts include cattle (*Bos taurus*), American bison (*Bison bison*), water buffaloes (*Bubalus bubalis*), and wisents (European bison; *Bison bonasus*). These intermediate hosts harbor sarcocysts (muscle cysts) that are infective when ingested by humans. Infective bradyzoites (dormant merozoite-like stages) are present in the sarcocysts. The bradyzoites penetrate the human intestinal epithelium and develop as sexual stages (macrogametocytes and microgametocytes) in cells in the lamina propria of the intestine. Fertilization occurs, and the oocysts sporulate in the lamina propria.

The sporocysts that are recovered in the stool are broadly oval and slightly tapered at the ends, measure 9 to 16 μm, and contain four mature sporozoites and the residual body (Table 23.2). Normally, two sporocysts are contained within the oocyst (similar to that seen with *C. belli*); however, in *Sarcocystis* infections, the sporocysts are released from the oocyst and normally are seen singly (Fig. 23.23). They tend to be larger than *Cryptosporidium* oocysts, which contain four sporozoites, so there should be no confusion between the two. The oocysts are fully sporulated when passed in the stool.

In humans, the prepatent period is 8 to 39 days, and patent infections can last as long as 18 months. *S. hominis* occurs on all continents, anywhere cattle or buffaloes have access to human feces and humans ingest raw or undercooked beef.

TABLE 23.18 *Sarcocystis* development and disease symptoms in humans[a]

Topic	Muscle	Intestine
Infection source	Water or food contaminated with infected feces containing oocysts from unknown carnivore or omnivore	Raw or poorly cooked meat
Infective stage	Oocyst or free sporocysts	Sarcocyst containing bradyzoites
Developmental stages	Intravascular schizonts (not seen); intramuscular sarcocysts	Sexual stages found in lamina propria; oocysts excreted in feces
Incubation time from ingestion of infective stage to disease symptoms	Weeks to months, lasting months to years	3–6 h, lasting approximately 36 h
Disease symptoms	Musculoskeletal pain, fever, rash, cardiomyopathy, bronchospasm, subcutaneous swelling	Nausea, anorexia, vomiting, stomach ache, bloat, diarrhea, dyspnea, and tachycardia; may also include abdominal pain and eosinophilia; severe symptoms may include segmental necrotizing enteritis (rare and not well confirmed)
Diagnosis	Biopsy specimen(s) contains sarcocysts; antibodies to bradyzoites; routine histology on biopsy specimens; wall may be PAS positive, but stain variability is common	Oocysts or sporocysts in feces, beginning about 5–12 days after ingestion of raw or undercooked meat; *S. hominis* (beef) is excreted 14–18 days after meat ingestion, while *S. suihominis* (pork) is excreted 11–13 days after ingesting meat
Therapy	Cotrimoxazole, furazolidone, albendazole, anticoccidials, pyrimethamine, anti-inflammatories	None

[a]Adapted from reference 51.

Sarcocystis suihominis. The life cycle is similar to that described above for *S. hominis*, with pigs serving as the intermediate hosts. The prepatent period is 9 to 10 days, and patency is generally longer than 36 days. *S. suihominis* presumably occurs on all continents where swine have access to human feces and humans ingest raw or poorly cooked pork.

Muscular *Sarcocystis* Infections. Muscular *Sarcocystis* infections in humans are usually subclinical or associated with mild clinical signs. Many of the cases have been incidental findings or associated with eosinophilic myositis. Muscular sarcocystosis has been associated with malignancies, primarily involving the tongue and nasopharynx. In these cases, the life cycle is a dead end, as no oocysts are passed in the feces.

Clinical Disease

Intestinal Disease. When humans serve as definitive hosts, prevention involves adequate cooking of beef, pork, and other infected meats (51, 52). In cases of intestinal sarcocystosis, infections are often asymptomatic and clear spontaneously. Occasionally, mild fever, diarrhea, chills, vomiting and respiratory problems may occur (Fig. 23.24).

Muscular Disease. When humans are intermediate hosts, preventive measures involve careful disposal of animal feces that may contain the infective sporocysts. This may be impossible in the wilderness areas, where wild animals may serve as reservoir hosts for many of the different types of organisms that in the past were grouped under the term *Sarcocystis* "*lindemanni.*" However, this name is no longer used. No specific therapy is known for the muscle stages of *Sarcocystis* spp. Various sizes of protozoan cysts, oocysts, and spores that occur in human stool specimens are given in Table 23.19.

When humans accidentally ingest oocysts from other animal stool sources, the sarcocysts that develop in human muscle apparently do little if any harm (schizogony) (51, 52). The prepatent period in humans is from 9 to 10 days. There is essentially no inflammatory response to these stages in the muscle and no conclusive evidence of pathogenicity of the mature sarcocyst. However, symptoms have been seen in some patients, probably associated with disin-

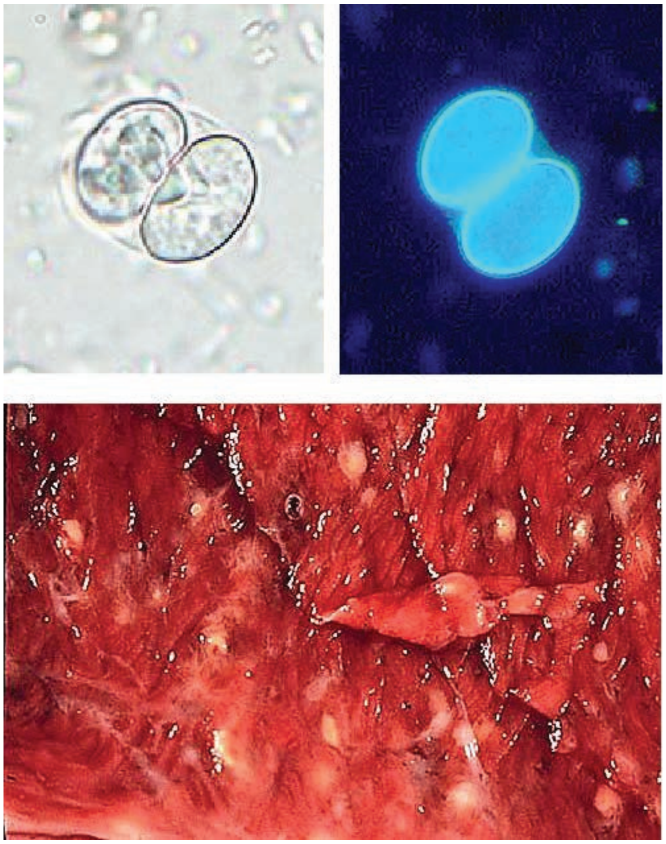

Figure 23.24 *Sarcocystis* spp. (Upper, left) Sporulated oocyst in unstained wet mount; (right) sporulated oocyst in wet mount viewed under UV microscopy, magnification ×400 (courtesy of the CDC Public Health Image Library). (Lower) Classic sarcocystosis in cut beef (courtesy of the University of Sydney, Centre for Veterinary Education, http://vetbook.org/wiki/cow/index.php/Sarcocystis_spp). doi:10.1128/9781555819002.ch23.f24

tegration of the sarcocysts (Table 23.18). Painful muscle swellings, measuring 1 to 3 cm in diameter, initially associated with erythema of the overlying skin in various parts of the body, occur periodically and last for 2 days to 2 weeks. Some patients also have fever, diffuse myalgia, muscle tenderness, weakness, eosinophilia, and bronchospasm. No apparent clinical manifestations have been found in patients in whom myocardial *Sarcocystis* infection was detected accidentally by autopsy. A number of different morphological types of skeletal and cardiac muscle sarcocysts have been recovered in humans. No specific therapy is known for this type of infection; however, corticosteroids should reduce the allergic inflammatory reactions occurring after cyst rupture.

Intact sarcocysts in skeletal or cardiac muscle of humans measure up to 100 by 325 µm and are usually not accompanied by an inflammatory reaction (Fig. 23.25). Each sarcocyst contains many bradyzoites, approximately 7 to 16 µm long. Inflammation follows disintegration of the cysts and death of the intracystic bradyzoites. Vasculitis is seen in the muscle and subcutaneous tissues. Histologic findings include myositis with vasculitis and sometimes myonecrosis.

Muscular Disease Outbreak. Prior to an outbreak from Tioman Island, Malaysia, in 2011–2012, <100 cases of intramuscular infection with *Sarcocystis* spp. had been reported in humans. Ninety-two persons attended a college retreat, which was held in a small hotel on the coast of Tioman Island, where all outdoor activities were conducted on the beach or in the ocean. In this outbreak, 89/92 patients were identified with onset of fever (94%), myalgia (91%), headache (87%), and cough (40%) ≤26 days upon return from the retreat. In persons who had a fever, the fever had a relapsing-remitting nature in 57% of patients. Some patients reported facial and calf muscle swelling and myalgias. Changes in mastication and masseter muscles were also seen. Typical sarcocysts (length ≈190 µm) were observed in cell cultures inoculated with the muscle tissue homogenates of one of the patients, and directly in the muscle tissue of another patient. DNA sequences obtained from 1,812-bp fragments were compared with fragments in GenBank by using BLAST (http://blast.ncbi.nlm.nih.gov/). Sequences matched 100% of those reported as *Sarcocystis nesbitti*, which has been reported in the skeletal muscles of *Macaca mulatta* monkeys. Although only two patients were confirmed to be acutely infected with *S. nesbitti*, it was likely that the remaining students and teachers in the group had the same infection. Almost everyone in the group had similar signs and symptoms, with onset of illness within days of each other (53).

Diagnosis

Intestinal Disease. Presumptive diagnosis is often based on the patient's symptoms, including a history of having ingested raw or poorly cooked meat. Confirmation of the diagnosis may depend on several stool examinations, some of which may reveal sporocysts. They are generally passed beginning 14 to 18 days after beef ingestion and about 11 to 13 days after pork ingestion. Some researchers think that the zinc sulfate fecal flotation method is preferred to the formalin-ethyl acetate sedimentation method for sporocyst and/or oocyst recovery (13). Sporocysts of *S. hominis* tend to be more elongated than those of *S. suihominis*, but the two may be difficult to differentiate.

Muscular Disease. Intramuscular infection would be suspected based on symptoms, including persistent myalgia, episodic weakness, subcutaneous nodules, dermatomyositis, eosinophilia, and elevated muscle creatinine kinase levels. Often these symptoms, coupled with a history of travel

TABLE 23.19 Protozoan cysts, oocysts, sporocysts, and spores seen in human feces[a]

Organism	Shape	Size (µm)	Features	Comments
Balantidium coli	Spherical to oval	50–70	Large macronucleus, thick wall	Although cilia are within the cyst wall, they are more difficult to see than the cilia surrounding the trophozoite form
Cystoisospora belli	Oval, more tapered ends	20–33 by 10–19	Generally immature oocysts passed, but some containing two sporocysts may be visible	Not infectious when passed; best diagnostic approach is wet mount of concentrated sediment; some recommend flotation rather than sedimentation concentration
Sarcocystis hominis				
Oocysts	Spherical	15–19 by 15–20	Contain 2 sporocysts, but oocyst wall appears to be missing	Generally infectious when passed
Sporocysts	Oval	15–19 by 8–10	Contain 4 sporozoites	Infectious
Sarcocystis suihominis				
Oocysts	Spherical	15–19 by 15–20	Contain 2 sporocysts, but oocyst wall appears to be missing	Generally infectious when passed
Sporocysts	Oval	15–19 by 8–10	Contain 4 sporozoites	Infectious
Entamoeba histolytica/ *E. dispar*	Spherical	12–15		Precyst contains single nucleus (generally), while mature cyst contains 4 nuclei and possible chromatoidal bars with rounded ends
Giardia lamblia	Spherical to oval	11–14	Contains 4 nuclei, curved median bodies, and linear axonemes	Some cysts appear to be found; important to focus up and down to see morphologic characteristics; infective when passed
Cyclospora cayetanensis	Spherical	8–10	May appear as "wrinkled cellophane"	Not infectious when passed
Cryptosporidium hominis and *C. parvum*	4–6	Nearly spherical	Sporozoites may be difficult to see; modified acid-fast stains recommended	Infectious when passed
Encephalitozoon spp. and *Enterocytozoon bieneusi*	1.5–3.5	Oval, mimic small yeast cells and/or bacteria	Horizontal or diagonal stripe (polar tubule) may be difficult to see in every spore	Infectious when passed

[a] Other pathogenic protozoa, *Dientamoeba fragilis* (very rare cyst forms) and *Blastocystis* spp., may overlap *Giardia* and *Entamoeba* spp. in size and may appear spherical to somewhat oval.

to or living in tropical locations, suggest the next step, which would be a muscle biopsy. Sarcocysts in biopsy specimens can be identified by microscopy of routine histologic sections stained with hematoxylin and eosin. Stain variability is common, and PAS positivity of the sarcocyst wall is not always seen.

Sarcocystis can be detected in meat by using routine histologic methods. Most sarcocysts in humans have been identified in skeletal and cardiac muscle; however, muscles in the larynx, pharynx, and upper esophagus have also been involved. Artificial digestion fluids can also be used on the meat source; after centrifugation, the sediment can be examined microscopically for the presence of bradyzoites.

Muscle sarcocystosis can be suspected in persons with signs and symptoms of myositis, with or without fever. Ill patients may have an elevated eosinophil count and may have an elevated creatine kinase (CK) with no other explanation for these findings. Eosinophil counts and CK levels may rise approximately 40 days after infection. A muscle biopsy may or may not demonstrate classic sarcocysts. Molecular testing is not widely available.

KEY POINTS—LABORATORY DIAGNOSIS

***Sarcocystis* spp.**

1. The oocysts are more easily recovered and identified in wet preparations (direct smear, concentration smear; flotation is recommended over sedimentation concentration methods).
2. The sporocysts are released from the oocyst and are seen singly (they are larger than *Cryptosporidium* oocysts).
3. Sarcocysts in muscle can be identified by routine histologic methods; however, there is a lot of staining variability (hematoxylin and eosin stains; PAS-positive sarcocyst walls may not be easily visible).

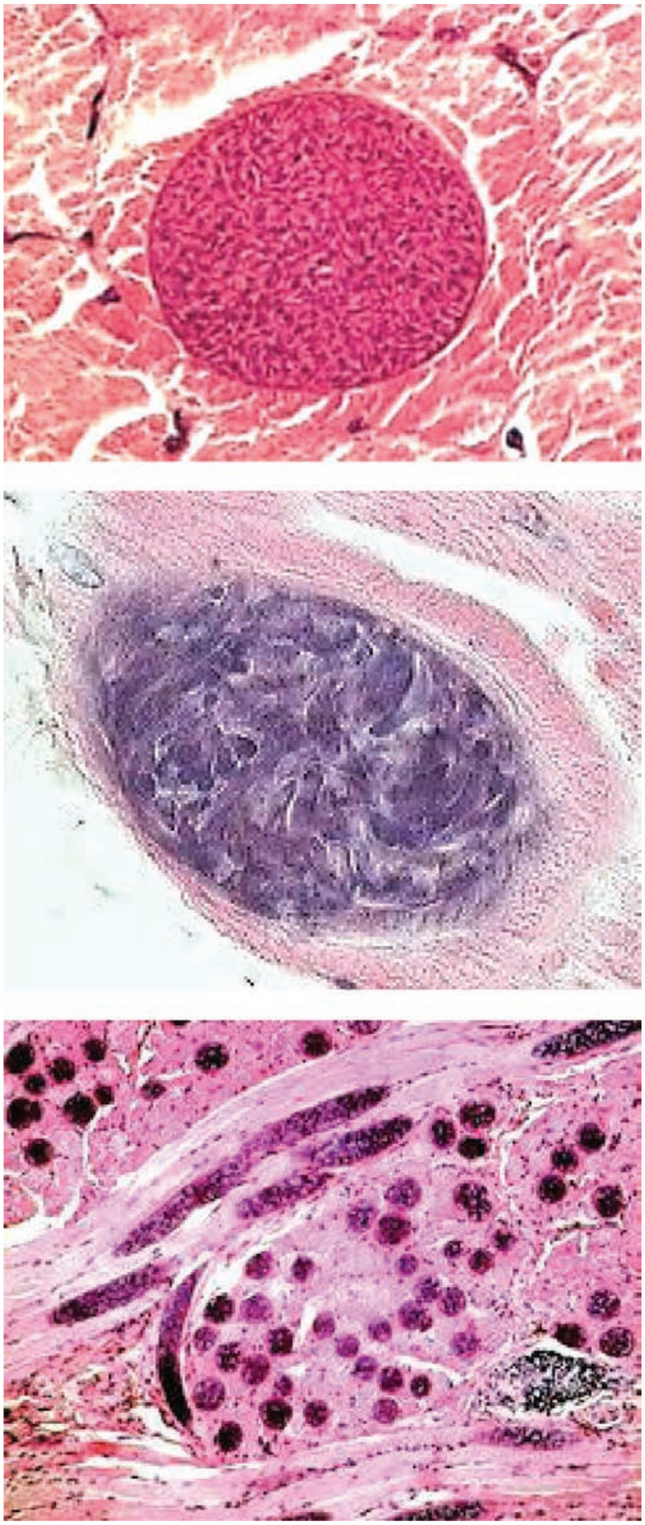

Figure 23.25 (Top) *Sarcocystis* spp. in human muscle. (Middle) Higher magnification showing individual elongate bradyzoites. (Bottom) Numerous sarcocysts in longitudinal and cross section in muscles of sheep tongue. Note the lack of direct inflammatory response to sarcocysts. (Bottom image courtesy of reference 51.) doi:10.1128/9781555819002.ch23.f25

Treatment

Intestinal sarcocystosis is self-limiting and often asymptomatic and lasts for a short time. Although cotrimoxazole and furazolidone have been used, their effectiveness is still questionable. Currently, no therapy has been approved for treatment for myositis, vasculitis, or related lesions in humans. The efficacy of albendazole has been reported but has not been demonstrated in controlled studies. Although drugs such as pyrimethamine and others used to treat toxoplasmosis have been considered, the efficacy of such drugs has not been documented. It is also unknown whether immunosuppressives might be effective in reducing the inflammatory reactions seen in vasculitis or myositis. Without more definitive data, no recommended course of therapy is currently available.

Epidemiology and Prevention

Apparently, multiple ruminant species serve as intermediate hosts of *Sarcocystis* spp. and as sources of infection for humans, and humans serve as intermediate hosts for several species of *Sarcocystis*, perhaps by ingesting sporocysts excreted by predators of nonhuman primates. Humans, baboons, and rhesus monkeys serve as definitive hosts for *S. hominis*, and humans, chimpanzees, and rhesus and cynomolgus monkeys serve as definitive hosts for *S. suihominis*. *S. hominis* occurs worldwide in areas where cattle or buffalo have access to human feces and humans ingest raw or undercooked beef. *S. suihominis* probably occurs worldwide in areas where swine have access to human feces and humans ingest raw or undercooked pork.

Thoroughly cooking or freezing meat to kill bradyzoites within the sarcocysts will prevent intestinal sarcocystosis. Freezing at –4 and –20°C for 48 and 24 h, respectively, is sufficient to kill bradyzoites. It has been documented that rare roast beef and hamburger purchased from a supermarket contained bradyzoites infectious for dogs whereas cooked products were not infectious for dogs.

Prevention of infection in food animals depends on eliminating the sporocyst stage from human feces in contaminated water, feed, and bedding. Also, to prevent humans from serving as intermediate hosts, ingestion of sporocysts must be prevented; the most likely source is contaminated water for drinking or washing foods. Boiling is the best method to guarantee disinfection. Thorough washing or cooking of foods is also recommended.

It has been reported that more than 27 outbreaks per year of food poisoning caused by consuming horse meat were reported in Kumamoto Prefecture (including Kumamoto City), Japan, from January 2009 to September 2011. It was found that the causative agent of the outbreaks was a protein that had originated from bradyzoites of *Sarcocystis fayeri* parasitizing the horse meat. After public health recommendations to freeze the meat at –20°C for

48 h or more, no subsequent cases were reported from the ingestion of raw horse meat containing *S. fayeri* (54).

Microsporidia

Although the microsporidia are true eukaryotes (i.e., they possess a typical eukaryotic nucleus, endomembrane system, and cytoskeleton), they also display molecular and cytological characteristics of prokaryotes, including features of the translational apparatus and genome size (range of bacteria), and lack recognizable mitochondria, peroxisomes, and typical Golgi membranes. These unusual features are thought to reflect their primitive eukaryotic nature. However, it is now well recognized that these unicellular organisms are best described as representing one of many offshoots of the fungal kingdom. Phylogenetic studies have confirmed that the microsporidia evolved from the fungi, being most closely related to the zygomycetes (55, 56). Other features that are shared with fungi include the presence of chitin and trehalose, similarities in cell cycles, and certain gene organizations. Microsporidia are now considered to be highly derived fungi that underwent genetic and functional losses, thus resulting in one of the smallest eukaryotic genomes known. However, at this point, clinical and diagnostic issues and responsibilities may remain with the parasitologists, and these organisms will be maintained as a part of this parasitology text. We may be in a transition stage, similar to that seen with *Pneumocystis jirovecii* as it was moved from the parasites to fungi in terms of classification status (Tables 23.20, 23.21, and 23.22).

During the early part of the 19th century, an epidemic disease was causing tremendous problems in the silkworm industry, particularly in Italy and France; the disease was called pébrine, which means "pepper disease." In 1863 Louis Pasteur was asked to study this problem, and by 1870 he had published his findings, in which he described a method for controlling and preventing the disease. This work represents the first scientific study of a disease caused by a protozoan, a microsporidian parasite, which resulted in practical control measures. Nägeli named the agent *Nosema bombycis*; however, he placed it in the Schizomycetes (at that time consisting of yeast and bacteria). A similar infection is seen in honeybee hives and is caused by *Nosema apis*. In 1882, Balbiani suggested the order Microsporida to handle *N. bombycis*, the only microsporidian known at the time. It was not until 1976 that the phylum Microspora was created, and it was updated in 1992 to propose that Microsporidia Balbiani 1882 should be the acknowledged phylum name, as well as the correct author and date (56). Currently, the phylum contains more than 170 genera and approximately 1,400 species, many of which are of medical, veterinary, and agricultural importance.

The microsporidia are obligate intracellular organisms that have been recognized in a variety of animals, particularly invertebrates. Typical sizes range from 1.5 to 5 μm wide and 2 to 7 μm long; unfortunately, the organisms found in humans tend to be quite small, ranging from 1.5 to 2 μm long. Until recently, awareness and understanding of human infections have been marginal; only with increased understanding of AIDS within the immunosuppressed population has attention been focused on these organisms. Limited availability of EM capability has also played a role in the inability to recognize and diagnose these infections. However, with the introduction of newer diagnostic methods, the ability to identify these parasites has definitely improved. The organisms are characterized by having spores containing a polar tubule, which is an extrusion mechanism for injecting the infective spore contents into host cells. To date, several genera have been recognized in humans: the more common, *Encephalitozoon* and *Enterocytozoon*, and the less common, *Brachiola*, *Pleistophora*, *Trachipleistophora*, *Vittaforma*, *Septata*, *Anncaliia*, *Tubulinosema*, and "Microsporidium," a catch-all genus for organisms that have not yet been classified (or may never be classified due to a lack of specimen). Classification criteria include spore size, configuration of the nuclei within the spores and developing forms, the number of polar tubule coils within the spore, and the relationship between the organism and host cell.

Microsporidiosis is an important emerging opportunistic infection in HIV-infected patients, and many patients with chronic diarrhea have been seen worldwide. Patients infected with *Cryptosporidium* spp. may also have concurrent infections with microsporidia, and this number may approach 30%. These findings emphasize the importance of considering both organisms as potential causative agents of diarrhea in compromised patients, particularly those with HIV. However, with the current use of antiretroviral combination therapy, the same decrease seen with cryptosporidiosis in this patient group has also been seen with microsporidiosis. Microsporidia also cause disease in organ transplant recipients, children, travelers, contact lens wearers, and the elderly. Microsporidiosis may involve any tissue of the body, including muscle, intestine, gallbladder, liver, kidneys, eyes, brain, lungs, skin, and nasal sinuses. Intestinal microsporidiosis is the most common, occurring in 30% to 50% of AIDS patients with chronic diarrhea.

Life Cycle and Morphology

The spore is the only life cycle stage able to survive outside of the host cell and is the infective stage. The spore normally reaches the new host through ingestion, although other routes of transmission have been identified. The life cycles of microsporidia infecting humans have three

TABLE 23.20 Microsporidia causing human infection[a]

Species	Nonhuman hosts	Sources of human infection	Sites of infection	Comments
Common				
Enterocytozoon bieneusi	Pigs, nonhuman primates, nonmammals (chickens, pigeons)	Fecal-oral; oral-oral, inhalation, ingestion, direct human-to-human; organ transplants	Small intestine, gallbladder, liver, disseminated (respiratory system)	Short-term culture only; 3 strains identified but not named; AIDS patients with chronic diarrhea, immunocompetent individuals; more than 90 genotypes described (17 infect humans); zoonotic and nonzoonotic genotypes; human isolates generally belong to group 1
Encephalitozoon cuniculi			Systemic, eye, respiratory system, urinary tract, liver, peritoneum, brain	
Strain I (rabbit)	Rabbits, rodents, carnivores, monkeys	Probably ingestion	Cerebrospinal fluid, intestine, respiratory, kidneys, nasal epithelium, eyes	Definitely zoonotic; spores highly resistant in environment (months under humid conditions); waterborne infections feasible
Strain II (mouse)	Mice, wild rats, blue foxes	Not relevant	Not relevant	Not identified in humans
Strain III (dog)	Dogs, tamarins (may have been imported from United States)	Probably ingestion	Kidneys, adrenal glands, ovaries, brain, heart, spleen, lungs, lymph nodes	Case occurred in Italy; AIDS (may be first report of infection involving ovaries); identified in humans in United States (not confirmed in dogs related to cases)
Encephalitozoon hellem	Psittacine birds	No epidemiologic studies; surface waters not confirmed; probably ingestion, aerosols, or oral or ocular direct inoculation; for drug addicts, contaminated syringes suggested but not proven	Disseminated; ocular in HIV-positive patients (patients with eye infections owned birds); respiratory tract, urinary tract, systemic	Asymptomatic respiratory infections reported; bronchoalveolar lavage in HIV-seronegative patients; stools from travelers (coinfected with *E. bieneusi*); *E. hellem* isolates from the United States and Europe are different populations
Encephalitozoon intestinalis	Dogs, donkeys, pigs, cows, goats, free-ranging gorillas	Probably ingestion, inhalation, direct inoculation; waterborne transmission suspected	Small intestine, disseminated; immunocompromised and immunocompetent, biliary tract, respiratory tract, bone, skin, systemic	Second most common species infecting humans; double infections with *E. bieneusi* occur
Uncommon				
Anncaliia (Brachiola, Nosema) algerae	Mosquitoes	Possible arthropod-borne	Eyes, muscle; possible dissemination; immunocompromised and immunocompetent	
Anncaliia (Brachiola, Nosema) connori	None identified	Unknown	Systemic	Formerly *Nosema connori*; immunocompromised child (SCID)
Anncaliia (Brachiola, Nosema) vesicularum	None identified	Unknown	Corneal stroma, skeletal muscle	Probably a *Nosema*-like species; non-HIV patient
Microsporidium (Nosema) africanum	None identified	Unknown	Corneal ulcer of African woman	Catch-all genus for organisms that could not be identified; HIV-seronegative individual at autopsy
Microsporidium (Nosema) ceylonensis	None identified	Unknown	Corneum	Boy gored by goat (injured eye), but may not be relevant

(continued)

TABLE 23.20 Microsporidia causing human infection[a] *(continued)*

Species	Nonhuman hosts	Sources of human infection	Sites of infection	Comments
Pleistophora ronneafiei	Fish, reptiles; possible animal reservoir not defined	Unknown	Muscle	Non-HIV and HIV positive; *Pleistophora* spp. (2 cases) may actually be *Trachipleistophora* spp.
Trachipleistophora anthropophthera	None identified, but may be insects	Unknown	Brain, heart, kidneys, eyes	AIDS patients
Trachipleistophora hominis	None identified, but may be insects	Unknown, but probably direct inoculation	Muscle, nasal epithelium, conjunctiva, keratitis	AIDS patient (severe sinusitis, myositis); immunocompetent patient
Vittaforma corneae (*Nosema corneum*)	Appear to be ubiquitous	Waterborne very likely; some intestinal infections may reflect intestinal passage of spores rather than infection	Eyes, corneal stroma; urinary tract	First human isolate established in an *in vitro* culture; non-HIV patient; formerly *Nosema corneum*
Tubulinosema acridophagus	Grasshoppers	Unknown	Systemic	From patient with chronic lymphocytic leukemia and acute renal failure

[a] Adapted from references 55, 56, 59, 60.

phases: infective, proliferative, and sporogonic (Fig. 23.26 and 23.27).

Infective Stage. As seen in Fig. 23.26, infection occurs with the introduction of infective sporoplasm through the polar tubule into the host cell. The sporoplasm passes through the tubule and is deposited into a new host cell in less than a second, thus initiating infection within a new host cell.

Proliferative Stage. The proliferative phase is initiated when a sporoplasm grows and divides extensively, producing many organisms within the new host-cell cytoplasm.The life cycle includes repeated divisions by binary fission (merogony) or multiple fission (schizogony) and spore production (sporogony). Both merogony and sporogony can occur in the same cell at the same time.

Sporogonic Stage. During sporogony, a thick spore wall is formed, providing environmental protection for this infectious stage of the parasite. An example of infection potential is illustrated by *Enterocytozoon bieneusi*, an intestinal pathogen. The spores are released into the intestinal lumen and are passed in the stool. These spores are environmentally resistant and can then be ingested by other hosts. There is also evidence for inhalation of spores and evidence in animals that suggests that human microsporidiosis may also be transmitted via the rectal route (57).

While the spore structures themselves are characteristic of microsporidia, the number of spores produced in sporogony, the way they are produced, and the host-parasite interface will differ among the microsporidian genera. The host-parasite interface may involve (i) direct contact with host-cell cytoplasm, (ii) indirect contact with host-cell cytoplasm by production of a parasite-secreted envelope (sporophorous vesicle), (iii) indirect contact by production of a parasite-induced, host-produced envelope (parasitophorous vacuole), or (iv) indirect contact by a host-produced envelope (parasitophorous vacuole) and parasite-produced secretions. These characteristics, along with morphologic features of developmental stages, nucleation, site of infection, and serologic and molecular features, help to identify the genus and species (55) (Table 23.21).

Clinical Disease

Microsporidia were recognized as causing disease in animals as early as the 1920s but were not recognized as agents of human disease until the AIDS pandemic began in the mid-1980s. Several earlier human cases had been reported but were thought to be very unusual. Currently, with more than 1,300 species of microsporidia, one would expect confirmation of additional human parasites in the future (Table 23.20) (57, 58).

TABLE 23.21 Host-parasite interface among the microsporidia genera[a]

Type of interface	Description and examples
Type I. Direct contact	Direct contact with host-cell cytoplasm *Nosema*, *Brachiola*, *Anncaliia*, *Enterocytozoon*
Type II. Indirect contact; host-produced isolation	A. Parasitophorous vacuole: host forms single membrane surrounding the developing parasite cell cluster. This is present during the proliferative and the sporogonic phases; however, the parasite relationship to this membrane undergoes change. The developing parasite cells maintain a very close relationship with this envelope until they develop the thickened sporont plasmalemma, then they appear loose within the vacuole. *Encephalitozoon cuniculi*.
	B. Host ER[a]: double membrane surrounds parasite cells throughout development. In the proliferative phase, the host ER double membranes follow the plasmalemma of the dividing cells so that no obvious vacuole is formed. In sporogony, the host ER does not divide with the sporonts but forms a double-membrane parasitophorous vacuole surrounding the cluster of organisms formed in sporogony. *Vittaforma* sp.
Type III. Indirect contact; parasite-produced isolation	A. Parasite-secreted envelope surrounds parasite cells throughout development. It becomes a sporophorous vesicle in sporogony when the parasite plasmalemma pulls away from the secreted envelope and then the plasmalemma thickens. *Pleistophora* sp.
	B. The parasite develops in direct contact with the host cell cytoplasm during early development but then a parasite-formed membrane isolates the sporonts from host cytoplasmic contact. *Vairimorpha* sp.
Type IV. Indirect contact; host- and parasite-produced isolation	A. Host ER closely aligns itself against the parasite plasmalemma in merogony. Then the parasite produces “blisters” arising from the plasmalemma to form the interfacial envelope. Thus a sporophorous vesicle is formed in sporogony and may also contain tubules. *Loma* sp. and *Glugea* sp.
	B. The host and parasite contribute to the formation of a thick interfacial envelope that surrounds all stages of parasite cells. *Trachipleistophora* sp.
	C. Host-formed parasitophorous vacuole surrounds the parasite cluster, and parasite-secreted material surrounds each parasite cell inside the parasitophorous vacuole. *Encephalitozoon intestinalis*

[a] Adapted from reference 55.
[b] ER, endoplasmic reticulum.

***Enterocytozoon bieneusi*.** *Enterocytozoon* was the first genus of microsporidia created for a human infection. Primarily, the organism infects the apical portion of enterocytes of the small bowel and produces a slight flattening of the epithelium (Fig. 23.28). Villus atrophy occurs due to rapid desquamation of infected enterocytes.The parasite can disseminate to the epithelial lining of the common bile duct, gallbladder epithelium, and biliary and respiratory tracts. *E. bieneusi* has been documented most often in male AIDS patients, causing malabsorption resulting in chronic diarrhea, and has been associated with AIDS-related sclerosing cholangitis. This microsporidian has also been documented in HIV-negative individuals with and without diarrhea and has been found in tracheal, bronchial, and nasal epithelium. *E. bieneusi* can also be found in stool samples after staining with modified trichrome.

TABLE 23.22 Microsporidia: general information

Genus	Body site	Diagnosis	Comments
Anncaliia, *Brachiola*, *Encephalitozoon*, *Enterocytozoon*, *Microsporidium*, *Pleistophora*, *Septata*, *Trachipleistophora*, *Vittaforma*, *Tubulinosema* (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most common pathogenic species recovered in humans)	All body organs, including eyes	Routine histology with hematoxylin and eosin (fair); tissue Gram, acid fast, PAS, silver recommended (spores); other specimens (stool, urine, etc.), modified trichrome stains, optical brightening agents, poly- or monoclonal antibody-based kits (FA, EIA); animal inoculation not recommended—laboratory animals may carry occult infections; EM may be required; some can be cultured, but results are not consistent among the various genera and species	These organisms have been found as insect or other animal parasites; route of infection may be ingestion, inhalation, or direct inoculation (eye); well documented as emerging opportunistic infection in AIDS and other immunocompromised patients; name changes and reclassifications continue to occur; also refer to Table 23.20

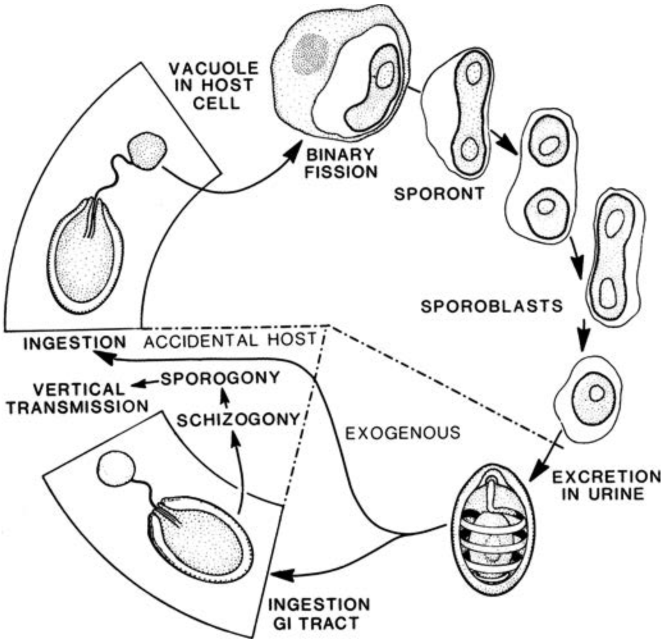

Figure 23.26 Life cycle of microsporidia. (From Gardiner CH et al, *An Atlas of Protozoan Parasites in Animal Tissues*, U.S. Department of Agriculture handbook no. 651, U.S. Department of Agriculture, Washington, DC, 1988.) doi:10.1128/9781555819002.ch23.f26

More than 90 *E. bieneusi* genotypes have been reported, with new genotypes continuing to be described. Nearly all of the genotypes that have been shown to infect humans so far belong to group 1. Currently, human infections by *E. bieneusi* isolates that cluster outside group 1 are exceptional and have been described in a single recent study performed in Central Africa.

Immunocompromised patients. Numerous cases of infection with *E. bieneusi* have been found in AIDS patients. Chronic intractable diarrhea, fever, malaise, and weight loss are symptoms of *E. bieneusi* infections and are similar to those seen in patients with cryptosporidiosis or isosporiasis. These patients have previously been diagnosed with AIDS, and they tend to have four to eight watery, nonbloody stools each day, which can be accompanied by nausea and anorexia. There may be dehydration with mild hypokalemia and hypomagnesia, as well as D-xylose and fat malabsorption. The patients tend to be severely immunodeficient, with a CD4 count usually below 200 and often below 100. Dual infections with *E. bieneusi* and *Encephalitozoon intestinalis* that infects the small intestine have also been reported. However, demonstration of *E. bieneusi* spores in extraepithelial tissues does not always appear to be associated with development of subsequent systemic infections.

E. bieneusi spores have been identified in sputum and bronchoalveolar lavage fluid, as well as stool samples. Although no pulmonary pathology can be established in all cases, it is well established that *E. bieneusi* is capable of colonizing the respiratory tract, and these clinical specimens may reveal the presence of spores. Using transmission EM, multiorgan microsporidiosis due to *E. bieneusi* has been diagnosed in some HIV-infected patients; organisms can be recovered in stools, duodenal biopsy specimens, nasal discharge, and sputum.

Recently, a new and highly divergent *E. bieneusi* genotype has been discovered in a human host (renal transplant patient); this genotype can be grouped with an equid species (59). Although the patient became sick 7 years post-transplantation, symptoms began soon after contact with water contaminated with horse stool. These findings should be taken into account for the management of immunocompromised patients such as HIV-infected patients and solid-organ transplant recipients. In these types of high-risk patients, they should be advised to avoid close contact with animals and follow prophylactic measures that may prevent infection.

Immunocompetent individuals. Infection with this organism has also been found in individuals with intact immune function; symptoms tend to be self-limited, and the diarrheal disease resolves within 2 weeks. These cases suggest that *E. bieneusi* may be more commonly associated with sporadic diarrheal disease than was previously suspected and that the immune system may play a role in the control of this organism within the intestine. It is also quite possible that *E. bieneusi* may persist as an asymptomatic infection in immunocompetent individuals.

Spore description. Finding several polar filaments within a multinucleate plasmodium by EM is diagnostic for *Enterocytozoon*. The development of multiple polar tubules within a multinucleate parasite cell is a unique feature of the developmental cycle of *Enterocytozoon*. The plasmodium divides by multiple fission, producing a dozen or more sporoblasts which mature into spores, all in direct contact with the host-cell cytoplasm. Spores measure 1.3 µm by 0.8 µm and contain a single nucleated sporoplasm surrounded by approximately six polar tubule coils, arranged in a double row (55).

***Encephalitozoon* spp.** Both *Encephalitozoon cuniculi* and *Encephalitozoon hellem* have been isolated from human infections, the first species from the central nervous system and the second from the eye. The first case occurred in a 9-year-old Japanese boy who presented with neurologic symptoms such as convulsions, vomiting, headaches, fever, and periods of unconsciousness. The spores were recovered in the cerebrospinal fluid and urine. This particular patient was treated with sulfisoxazole and recovered. Another case of *E. cuniculi* was in a 2-year-old child with a similar neurologic illness; the spores were recovered from the urine.

Immunocompromised patients. A case of keratoconjunctivitis and chronic sinusitis due to infection with *E. cuniculi* in an AIDS patient has been reported; diagnosis was confirmed by EM. This species has been found in the liver, as well as peritoneal, cerebral, and disseminated infections in AIDS patients. Several eye infections with *E. hellem* have been found in AIDS patients, including the first reported case of *E. hellem* infecting not only sinuses and conjunctivae but also the nasal epithelium. Overall, this species has been documented in disseminated infections and infection of the sinuses, nasal tissue, tongue, respiratory system, kidneys, and male genital tract. In the first case of disseminated *E. hellem* infection in an AIDS patient, a complete autopsy revealed organisms in the eyes, urinary tract, and respiratory tract. The presence of numerous organisms within the lining epithelium of almost the entire length of the tracheobronchial tree was very suggestive of respiratory acquisition. It is also interesting that although *E. cuniculi* and *E. hellem* differ biochemically and immunologically, their fine structure and development are indistinguishable. Some of the eye infections have also suggested a topical route rather than dissemination. Dual infection with *E. hellem* and *Vittaforma corneae* has also been found in an AIDS patient; this is the second isolation of *V. corneae* and the first description of urinary tract infection due to *V. corneae* in a patient with AIDS.

Spore description. The genus *Encephalitozoon* is characterized by a parasitophorous vacuole that surrounds the developing parasites and isolates them from the host-cell cytoplasm. Developing stages may contain one or more nuclei but the nuclei are not attached to each other. Multinucleate cells are long and narrow, not plasmodial. Proliferative cells usually break free of the vacuole membrane in sporogony. Sporonts form a thickened plasmalemma on individual cells. Within the parasitophorous vacuole, each

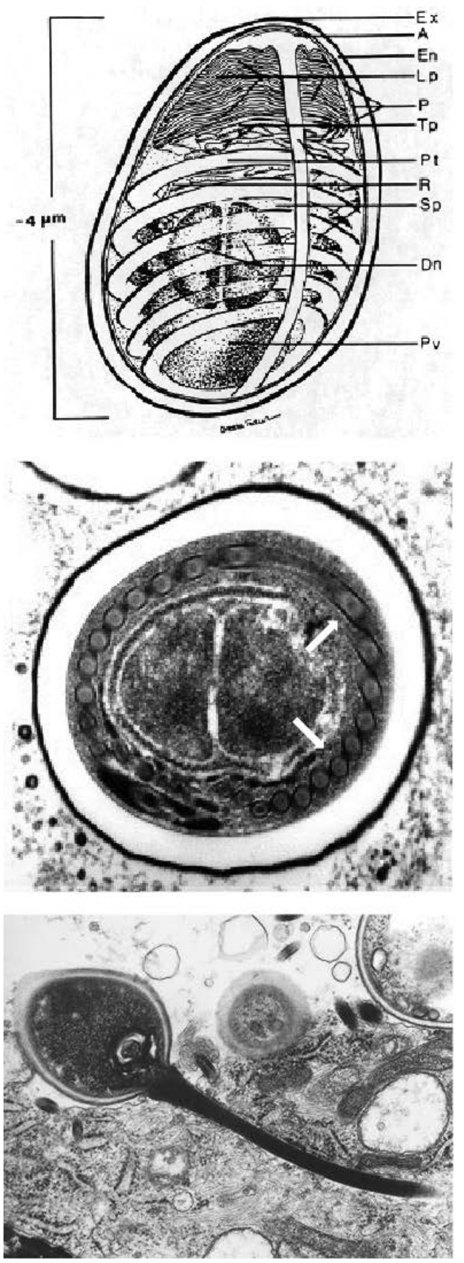

Figure 23.27 (Top) Diagram of the internal structure of a microsporidian spore. The spore coat has an outer electron-dense region called the exospore (Ex) and an inner, thicker, electron-lucent region, the endospore (En). A unit membrane (P) separates the spore coat from the spore contents. The extrusion apparatus—anchoring disk (A), polar tubule (Pt), lamellar polaroplast (Lp), spongiform polaroplast (Sp), and tubular polaroplast (Tp)—dominates the spore contents and is diagnostic for microsporidian identification. The posterior vacuole (Pv) is a membrane-bound vesicle, which sometimes contains a "membrane whirl," a "glomerular-like" structure, flocculent material, or some combination of these structures. The spore cytoplasm is dense and contains ribosomes (R) in a tightly coiled helical array. The nucleation may consist of a single nucleus or a pair of abutted nuclei, a diplokaryon (Dn). The size of the spore depends on the particular species and can vary from less than 1 µm to more than 10 µm. The number of polar tubule coils also varies from a few to 30 or more, again depending on the species observed. (Middle) EM of *Anncaliia* (*Brachiola*, *Nosema*) *connori* spore in adrenal gland showing the coiled polar filament (arrows) and two nuclei. Note the polar tubules are in a single row. (Magnification, ×30,000; courtesy of reference 55). (Bottom) Transmission electron micrograph of a microsporidian spore with an extruded polar tubule inserted into a eukaryotic cell. The spore injects the infective sporoplasm through its polar tubule. (Courtesy of the CDC Public Health Image Library; Massimo Scaglia, Laboratory of Clinical Parasitology, Institute of Infectious Diseases, University-IRCCS San Matteo, Pavia, Italy.)

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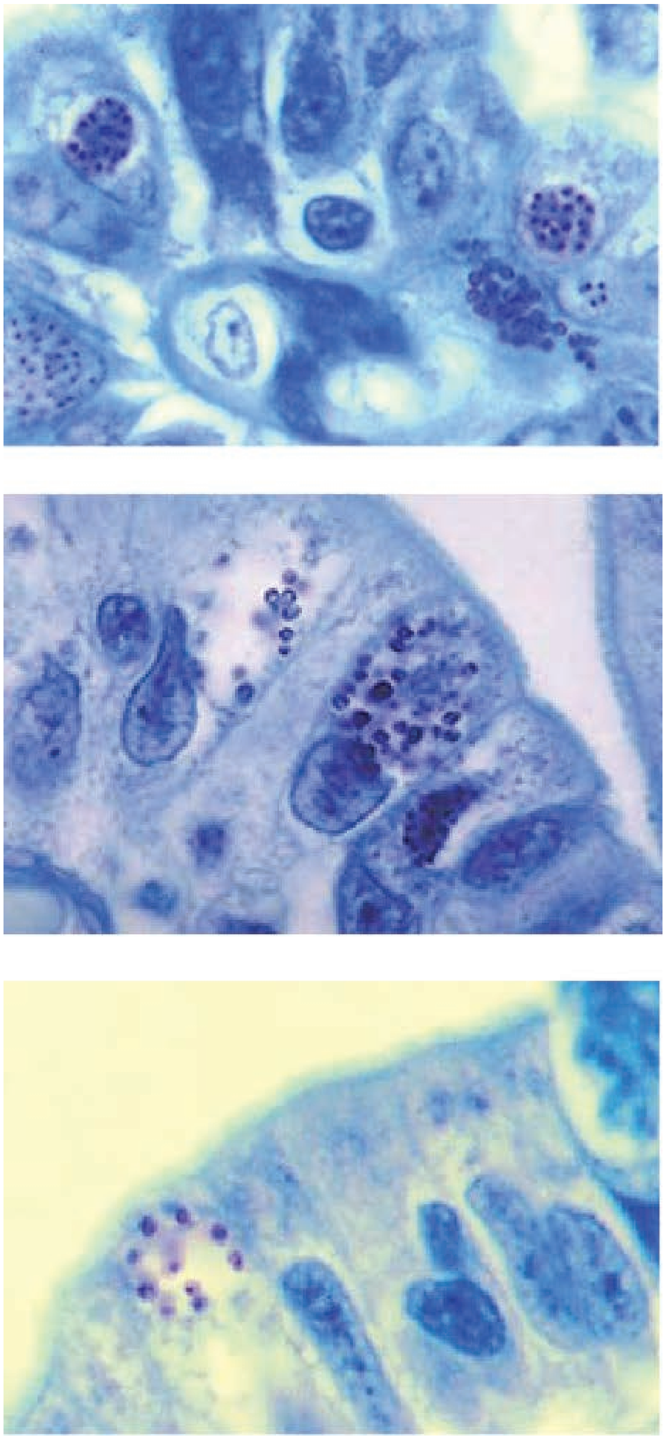


Figure 23.28 Spores in enterocytes. Note the position of the spores between the cell nucleus and the lumen of the intestine. Top and middle photographs taken with a 40× objective; bottom image taken with the 100× oil immersion objective. Note the dark-staining dot within the spores in the bottom photograph; this dot stains PAS positive. The tissue was stained with Giemsa stain. doi:10.1128/9781555819002.ch23.f28

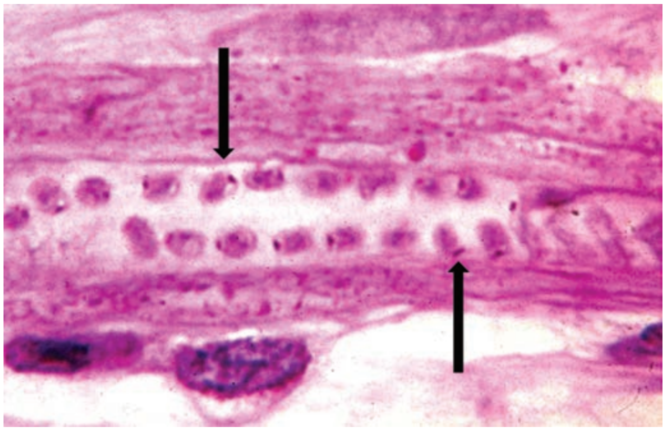
sporont elongates, divides, and produces spores. Spores measure 1 to 1.5 μm by 0.5 μm and contain a sporoplasm with a single nucleus and approximately six polar tubule coils arranged in a single row. *E. hellem* is morphologically the same as *E. cuniculi*, although the parasitophorous vacuole is not always present (55).

Encephalitozoon (Septata) intestinalis. *Encephalitozoon* (*Septata*) *intestinalis* infects primarily small intestinal enterocytes, but infection does not remain confined to epithelial cells. *E. intestinalis* is also found in lamina propria macrophages, fibroblasts, and endothelial cells. Dissemination to the kidneys, lower airways, and biliary tract appears to occur via infected macrophages (57). Fortunately, these infections tend to respond to therapy with albendazole, unlike the infections with *E. bieneusi*. Infections have been reported in the liver, renal system, colon, gallbladder, lungs, sinuses, and conjunctiva. It has been misdiagnosed as *E. bieneusi* in the intestine and as *E. hellem* in the eye, sinuses, and urine.

Spore description. Encephalitozoon (*Septata*) *intestinalis* has parasite-secreted material surrounding the developing stages and spores inside the parasitophorous vacuole. Spores are 2 μm by 1.2 μm, with a single nucleus and four to seven (usually five) polar tubule coils arranged in a single row (55).

***Anncaliia (Brachiola, Nosema)* spp.** *Anncaliia* (*Brachiola*, *Nosema*) *connori* has been identified in human tissues, with the spores being oval, measuring approximately 2 by 4 μm, and having paired nuclei (Fig. 23.29). The polar tubule has about 11 coils arranged in one to three rows. The single human case occurred in a 4-month-old infant

Figure 23.29 Section of appendix showing *Anncaliia connori* spores in muscularis. The anterior end of the spore has a PAS-positive granule (arrows). (PAS, ×1,260; Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch23.f29



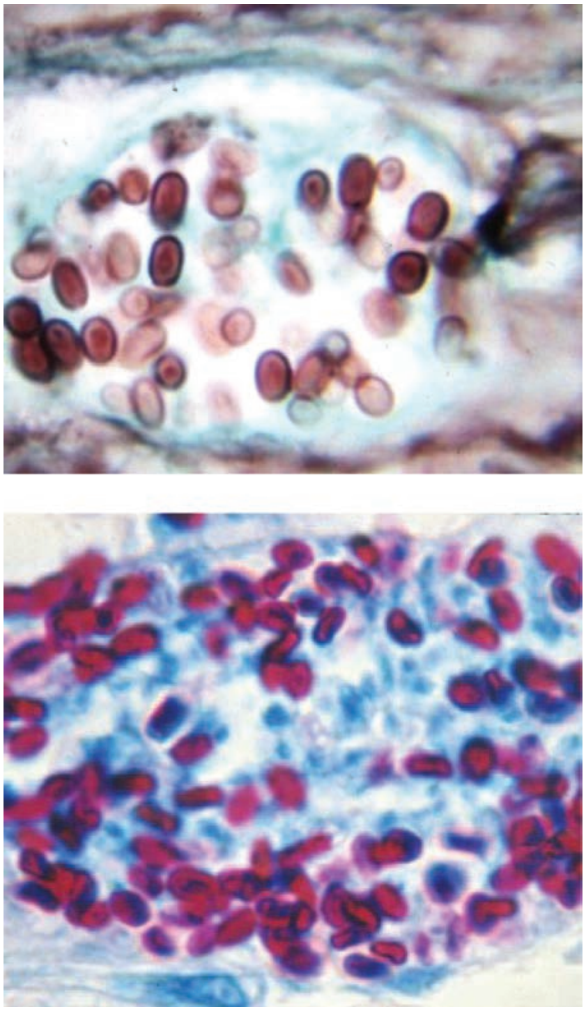


Figure 23.30 (Upper) *Anncaliia connori* spores in wall of ileum (90 min Grocott methenamine silver stain, ×1,300). (Lower) *A. connori* in myocardium (Ziehl-Neelsen stain, ×1,440). (Armed Forces Institute of Pathology photographs.)
doi:10.1128/9781555819002.ch23.f30

with combined immunodeficiency disease with a disseminated, fatal infection. Parasites were found in the myocardium, diaphragm, arterial walls, kidney tubules, adrenal cortex, liver, and lungs (Fig. 23.30). Concurrently, this patient also had *P. jirovecii* pneumonia.

Anncaliia (*Brachiola*, *Nosema*) *algerae* is apparently transmitted by mosquitoes. Infections have been found in the eye and muscle, possibly from a disseminated case. Both immunocompromised (HIV-negative, but receiving immunosuppressive therapy for rheumatoid arthritis) and immunocompetent patients have become infected. This organism has been known as an insect pathogen for some time. It appears that an initial infection of the eye allows the parasite to adapt to conditions within the mammalian host, thus spreading via macrophages to internal organs.

Brachiola vesicularum is probably a *Nosema*-like species found in non-HIV-infected patients. Corneal stroma and skeletal muscle were involved in a case in an AIDS patient. Although it was thought that *A. algerae* and *B. vesicularum* might be identical, there are ultrastructural differences between the two organisms. Thus, the designation of two species is currently accepted.

Tubulinosema sp. (closely related to *T. acridophagus*) myositis was documented in a patient with chronic lymphocytic leukemia and renal failure. This organism is similar to several species included in the genus *Nosema*, and *Anncaliia* has been classified within the same family. Since some of these organisms are well-known parasites of insects, the source of this particular infection was thought to probably be some type of insect (60).

***Microsporidium* spp.** *Microsporidium africanum* was identified in the histiocytes of a corneal ulcer. Although it was first thought to be a *Nosema* sp., EM confirmed the differences and it was designated *M. africanum*. No information on other hosts is available.

M. ceylonensis was found in an eye infection in a child who had been gored by a goat and suffered an eye injury. *Nosema helminthorum* was considered a possibility, since the organism is a parasite of goat tapeworms; however, since a generic classification could not be completed, it was assigned to the genus *Microsporidium*. Since no additional infections have been seen and there is no tissue left for further study, this genus name, *Microsporidium*, will probably remain. Since it is now accepted, you will see it written as *Microsporidium* rather than "*Microsporidium*."

Eye infections. Since 1990, hundreds of microsporidian keratoconjuctivitis cases have been reported, in both HIV negative and positive patients; however, only a few reports have identified the organisms to the specific genus level other than *Microsporidium*. Common features include follicular papillary conjunctivitis and coarse punctuate epithelial lesions evolving into keratitis before resolution. Additional clinical features include diffuse endotheliitis (inflamed corneal endothelium) with corneal edema and atopic keratoconjunctivitis (rare, affects young men with atopic dermatitis). Almost all of these cases resolved with the use of topical fluoroquinolone monotherapy (Fig. 23.31).

***Pleistophora* spp.** Microsporidia of the genus *Pleistophora* have rarely been identified in humans; however, three cases in immunocompetent patients have been reported. In these cases, atrophic and degenerating muscle fibers were full of spores, which were seen in clusters of about 12 organisms, each cluster enclosed by an enveloping membrane, the pansporoblastic membrane. The spores were oval, measuring approximately 2.8 by 3.4 μm. In one patient, the disease was characterized by a 7-month history of progressive gen-

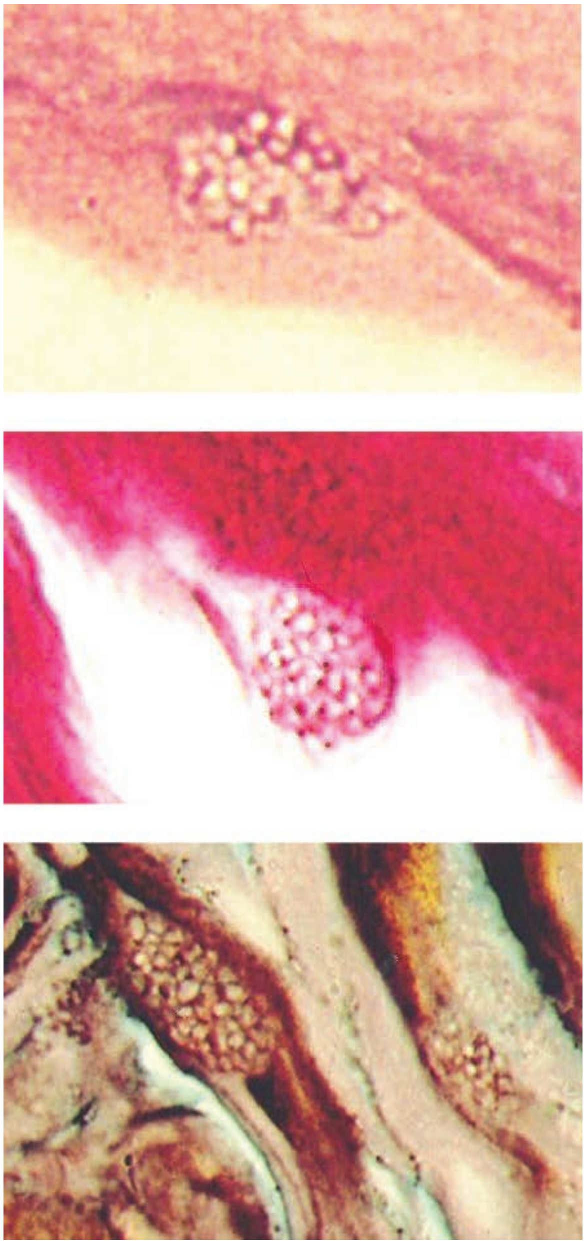


Figure 23.31 Microsporidian spores seen in an eye infection. (Top) Hematoxylin and eosin stain. (Middle) PAS stain; note the dark staining dot at the end of each spore. (Bottom) Silver stain; note spores are outlined. doi:10.1128/9781555819002.ch23.f31

eralized muscle weakness and contractions in addition to fever, generalized lymphadenopathy, and an 18-kg weight loss. Although the patient was diagnosed as having AIDS, the presence of HIV was never demonstrated. Early biopsies demonstrated atrophic and degenerating muscle fibers; later biopsies showed fibrosis and scarring. *Pleistophora* microsporidia have been identified in a confirmed HIV-positive patient. Ultrastructural studies of specimens from one of the cases led to the classification of *P. ronneafiei*; no information is available about possible animal reservoirs.

***Trachipleistophora* spp.** *Trachipleistophora hominis* causes severe myositis and sinusitis and was first detected in an AIDS patient. To date, no natural hosts other than humans have been identified, although when inoculated intraperitoneally into athymic mice, the organisms infect the skeletal muscle, as seen in human infections. *T. anthropophthera* is the second species in this genus to be described in humans and has been found in brain tissue of two AIDS patients at autopsy; the organisms were also found in the brain, heart, kidney, pancreas, thyroid, parathyroid, liver, bone marrow, lymph nodes, and spleen.

Vittaforma corneae. This organism has been documented in both immunocompromised (urinary tract) and immunocompetent (ocular) patients. These organisms are found everywhere, but no specific natural common hosts have been identified. PCR and sequencing data from surface water studies suggest that these organisms may be present in surface water and that they can also be present in human feces; it has been suggested that this might represent intestinal passage of microsporidial spores, although these patients were symptomatic with diarrhea (57).

Summary: Infections in Immunocompetent Hosts. In studies of rabbits and mice, the majority of immunologically competent hosts that become infected with microsporidia develop chronic and persistent infections with few clinical signs or symptoms. Spore shedding has been described as sporadic, and parasite replication seems to be regulated by the host immune system. However, if these animals are intentionally immunosuppressed, more severe symptoms are seen. On the basis of serologic studies, it is very likely that immunocompetent humans have persistent or chronic infections with microsporidia. This is also supported by the fact that patients with microsporidiosis who are HIV seronegative show clinical resolution of their infections after a few weeks. As improved diagnostic methods, such as PCR, are developed and used, verification of human subclinical microsporidial infections should become possible. Data strongly suggest that the true distribution of microsporidiosis in healthy humans is probably underestimated. Many eye infections with various species of microsporidia have been seen in immunocompetent patients; this possibility should always be considered when routine eye cultures show nothing clinically significant or in cases of corneal transplantation. Keratoconjunctivitis tends to be a self-limiting disease, while stromal keratitis is an ill-defined disease. Stromal keratitis is characterized by a slowly progressive course. The corneal picture resembles herpes simplex virus stromal keratitis or fungal keratitis cases, and is characterized by deep stromal infiltrates with overlying and surrounding stromal edema and keratic precipitates.

Summary: Infections in Immunocompromised Hosts. In the immunocompromised host, microsporidial infection may lead to overwhelming disease and death. The first cases of human microsporidiosis were identified in children with impaired immune systems, and infections have been widely recognized and studied in individuals with AIDS, primarily those with fewer than 100 $CD4^+$ T lymphocytes. Infection has also been recognized in organ transplant recipients who are intentionally immunosuppressed before and after transplantation; cases involve pediatric and adult kidney and liver transplant recipients, as well as those receiving bone marrow, lung, or heart-lung transplants. Microsporidiosis should always be considered in the differential diagnosis for a wide range of clinical syndromes in immunocompetent as well as immunocompromised individuals.

Diagnosis

Routine Histology. A number of techniques are available for recovery and identification of microsporidia in clinical specimens (Tables 23.22 and 23.23). Although the organisms have been identified in routine histologic preparations, they do not tend to stain predictably if at all. Occasionally, the spores take on a refractile gold appearance in formalin-fixed, paraffin-embedded, routine hematoxylin-and-eosin-stained sections. Some of the difficulty may be attributed to the use of formalin; alternative fixatives are optional. Spores are occasionally seen very well by using PAS stain, silver stains, Giemsa stain, or acid-fast stains. Modified Gram stains have also been found to be sensitive although not always standardized. The spore has a small, PAS-positive posterior body, the spore coat stains with silver, and the spores are acid-fast variable (Fig. 23.29 to 23.31). There is also evidence to indicate that specimens from plastic-embedded tissues are seen more easily, regardless of the fixative used. Tissue examination by EM techniques is still considered the best approach for identification to the genus/species levels (Fig. 23.32). However, this option is not available to all laboratories, and the sensitivity of EM may not be equal to that of other methods when examining stool or urine. Also, the amount of specimen sampled using EM is quite small and may result in a false negative if the appropriate infected tissue is not examined.

Techniques that do not require tissue embedding are now becoming more popular. Touch preparations of fresh biopsy material that are air dried, methanol fixed, and Giemsa stained have been used; however, screening must be performed at ×1,000 magnification. Another study, using cytocentrifugation followed by Giemsa staining, found that 27% of 55 AIDS patients with chronic diarrhea were positive for *E. bieneusi*; all results were confirmed by EM.

For histologic examination of tissue sections, glutaraldehyde fixation and plastic embedding are highly recommended, even if EM is unavailable. Parasites may be obscured by the host tissue in 6-μm paraffin-embedded preparations. Using light microscopy, 1-μm-thick plastic sections, heat-fixed to a glass slide and stained with toluidine blue, provide better visualization of microsporidia because of the thinness of the section. Plastic embedding provides a permanent tissue specimen that can be reevaluated by EM at a later time. Additionally, paraffin-embedded tissue can be deparaffinized and reprocessed for plastic embedding (55).

Routine Clinical Specimens. *Modified trichrome stains*. Other, more recent options recommend using modified trichrome stains in which the chromotrope 2R component added to the stain is 10 times the concentration normally used in the routine trichrome stain for stool (17, 57, 58) (Fig. 23.33). It is important to remember that the stool preparations must be very thin, the staining time must be 90 min, and the slide must be examined at ×1,000 (or higher) magnification. Unfortunately, there are many objects within stool material that are oval, stain pinkish with trichrome, and measure approximately 1.5 to 3 μm. If this stain is used for the identification of microsporidia in stool, positive control material should be available for comparison. Additional modifications of this method include the use of heat and a shorter staining time (17). There is also some evidence to indicate that pretreatment of fecal specimens (1:1) with 10% KOH may provide a better quality smear to examine when using the modified trichrome stains. Unfortunately, there are always situations in which artifact material can be confused with actual spores (Fig. 23.33).

Optical brightening agents. Another approach involves the use of chemofluorescent agents (optical brightening agents) such as calcofluor, Fungi-Fluor, or Uvitex 2B (Fig. 23.33). These reagents are sensitive but nonspecific; objects other than microsporidial spores will also fluoresce. This is a particular problem when examining stool specimens; both false-positive and false-negative results have been seen. When these reagents are used with other body fluids, particularly urine (generally cleaner specimen with less debris), the interpretation of results is much easier than when they are used with stool. There is now general agreement that this method is recommended as a simple, sensitive screening method for the detection of microsporidial spores in stool specimens.

Antigen detection. The newest approach for the identification of spores in clinical specimens uses antisera in an indirect FA procedure (Fig. 23.34). Fluorescing microspor-

TABLE 23.23 Microsporidia: recommended diagnostic techniques[a]

Technique	Use[b]	Comments
Light microscopy		
Stool specimens (more artifacts)		
Modified trichrome	++	Reliable, available; light infections difficult to identify
Giemsa	–	Not recommended for routine use; hard to read
Optical brightening agents	+	Calcofluor, Fungi-Fluor, Uvitex 2B; sensitive but nonspecific
IF technique	++	Commercial availability limits use; products in development
Other body fluids (fewer artifacts)		
Modified trichrome	++	Reliable, available; light infections difficult to identify
Giemsa	+	Urine, conjunctival swab, BAL, CSF, duodenal aspirate
Optical brightening agents	++	Calcofluor, Fungi-Fluor, Uvitex 2B; sensitive but nonspecific
IF technique	++	Commercial availability limits use; products in development
Routine histology		
Hematoxylin and eosin	+	Sensitivity uncertain with small parasite numbers
PAS	+	Controversy over effectiveness
Modified Gram stains (Brown Brenn, Brown-Hopps)	++	Sensitive, generally recommended
Giemsa	+	Sensitivity uncertain with small parasite numbers
Warthin-Starry	+	Not standardized, may not be necessary
Modified trichrome	++	Reliable, sensitive
IF technique	++	Commercial availability limits use; products in development
Electron microscopy		
Body fluids	+	Specific, sensitivity unknown; used for identification to species level
Tissue sections	++	"Gold standard" for confirmation, but sensitivity lower than for detection of spores in stool or urine; used for identification to species level
Serologic antibody detection	–	Reagents not commercially available, preliminary results controversial
Cultures	–	Generally used in the research setting; continued advances in culture options and organism survival/growth
PCR	–	Availability limited to research laboratories; studies ongoing

[a] IF, immunofluorescence detection procedure; BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.
[b] –, not available or recommended for routine use; +, reported; ++, techniques in general use (probably most widely used).

idial spores were distinguished by a darker cell wall and by internal visualization of the polar tubule as diagonal lines or cross lines within the cell. In another study with the same antiserum, 9 (30%) of 27 patients who had already been diagnosed as having cryptosporidiosis (8 AIDS patients and 1 non-AIDS but immunodeficient patient) were found to have *Encephalitozoon* spp. in the stool. Although there is some cross-reactivity with bacteria, this technique offers a more sensitive approach than routine staining methods currently available for the examination of stool specimens. Very specific reagents are in various stages of development and clinical testing and should provide some additional, more sensitive methods than are currently available at this time. Some of these reagents are also being used in conjunction with flow cytometry. As clinicians begin to suspect these infections and laboratorians become more familiar with the diagnostic methods, the number of positive patients, particularly those who are immunocompromised, may increase dramatically. Current recommendations also suggest that multiple diagnostic methods may be necessary to diagnose microsporidiosis, particularly when examining fecal specimens (17).

Molecular Methods. Molecular studies of the microsporidia have been limited to date (61–63). Few genes have been located, and DNA sequence studies have been reported for just a few of the identified human pathogens. Microsporidia have extremely small genomes, very similar to those seen in bacteria. This may be due to the early divergence of microsporidia or may be related to the highly adapted parasitic lifestyle of these organisms. Although PCR methods have been tried in the research setting, commercial products are not yet available. For HIV-infected patients suspected of having microsporidiosis but with negative stool and biopsy results, PCR testing of intestinal biopsy specimens may be helpful in diagnosing the infection. Application of molecular techniques to diagnosis, identification of isolates to the species level, and classification will

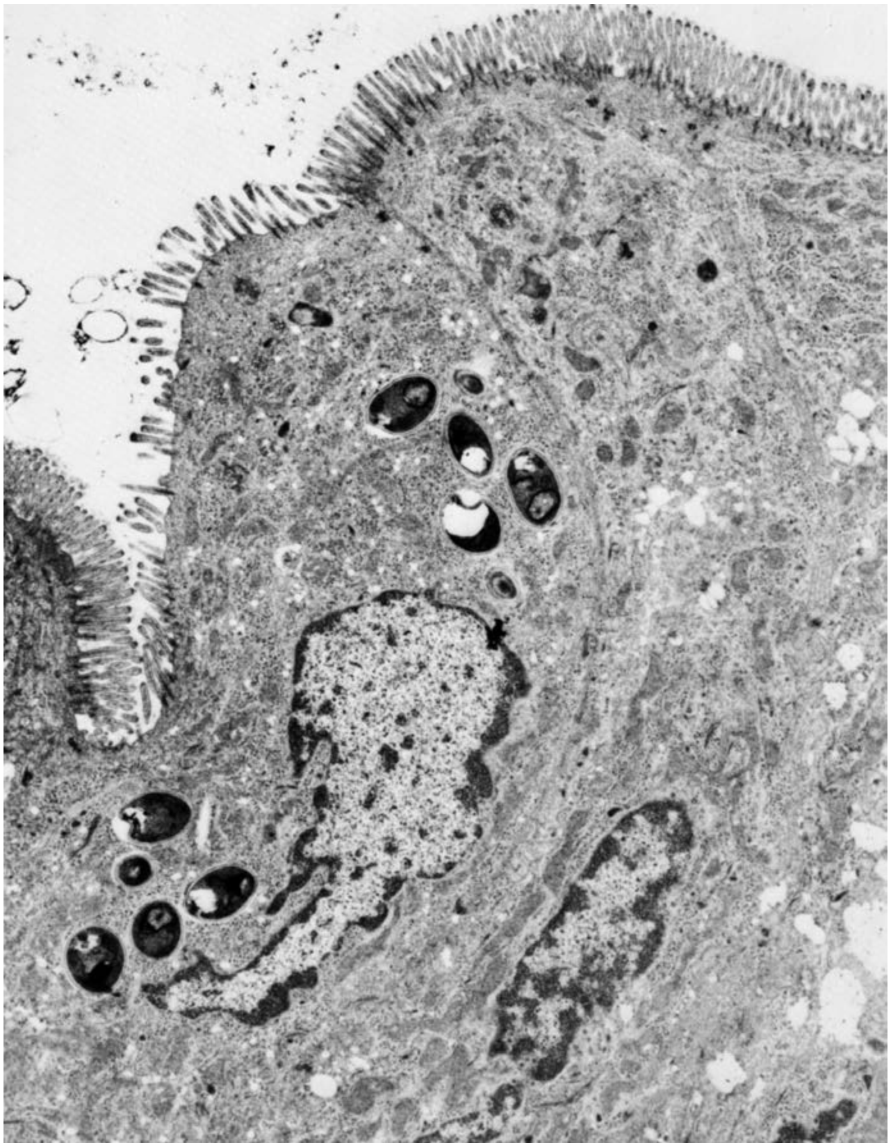


Figure 23.32 Spores in cytoplasm of intact jejunal enterocyte of a man with intractable diarrhea and malabsorption; transmission electron micrograph of a jejunal suction biopsy specimen. Note the dark oval developing spores; at higher magnification, some of the elements of the polar tubules would be visible. (Courtesy of Owen RL, *in* Farthing MJG, Keusch GI [ed], *Enteric Infection: Mechanisms, Manifestations and Management*, Chapman & Hall, Ltd., London, United Kingdom, 1987.)
doi:10.1128/9781555819002.ch23.f32

certainly continue to expand during the next few years. These advances should include newer diagnostic reagent kits that will simplify molecular testing in the routine clinical laboratory setting, particularly tests applicable to the stool specimen.

Several PCR-based methods to amplify different regions of small- and large-subunit rRNA genes are available for diagnosis of *E. bieneusi* and *E. intestinalis* infections; however, PCR inhibitors may interfere with the test. *In situ* hybridization can be performed for *E. bieneusi* in humans. Multiplex nested PCR for microsporidia is also available. The predominant species tend to be *E. bieneusi* or *E. intestinalis*.

Other diagnostic molecular methods include random amplified polymorphic DNA obtained by PCR, PCR-RFLP, and SDS-PAGE. A 75-kDa band on one-dimensional Western blot of *E. cuniculi* and *E. intestinalis* spore protein can be obtained using purified C1 Hsp70 protein. PCR amplification and species determination of microsporidia in formalin-fixed feces can also be performed after immunomagnetic separation (63).

Cell Culture. The use of *in vitro* culture methods continues to provide confirmatory as well as diagnostic information. This approach has been instrumental in the development of immunologic reagents for diagnosis and species differentiation. *In vitro* culture has also been used to assess the efficacy of antimicrobial agents on several microsporidian species including *Encephalitozoon cuniculi*, *E. hellem*, and *E. intestinalis* (57). However, the use of cell culture in routine clinical diagnosis is not practical and is generally

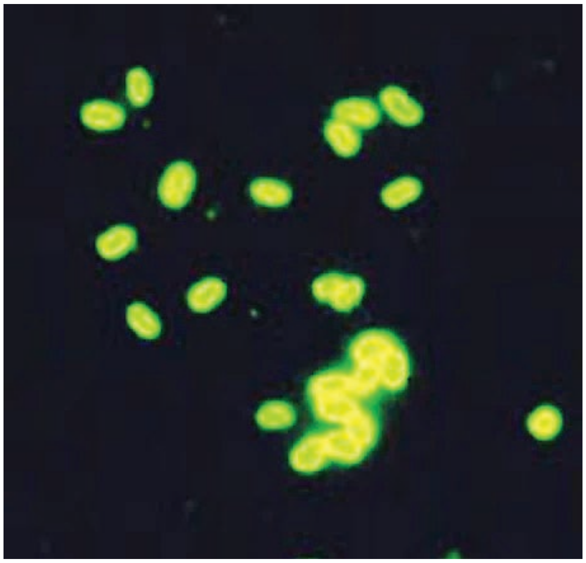


Figure 23.34 Microsporidian spores. This image shows a positive FA test using *Encephalitozoon*-specific reagent. Note the fluorescing spores, indicating the organisms are within the genus *Encephalitozoon*. doi:10.1128/9781555819002.ch23.f34

reserved for research facilities. A number of cell lines have been used, including monkey and rabbit kidney cells (Vero and RK13), human fetal lung fibroblasts (MRC-5), MDCK cells, and various fish and insect cells.. Although a number of the microsporidia have been cultured using these techniques, one of the most common human microsporidial pathogens, *E. bieneusi*, has been propagated only in short-term cultures (6 months); however, the use of salmonid leukocytes appears to support the development of this species. Difficulties encountered in growing this organism in continuous culture may reflect additional nutritional requirements that have not yet been identified.

Serologic Testing. A variety of serologic tests (carbon immunoassay, indirect immunofluorescent-antibody test, enzyme-linked immunosorbent assay, counterimmunoelectrophoresis, and Western blotting) have been used to detect

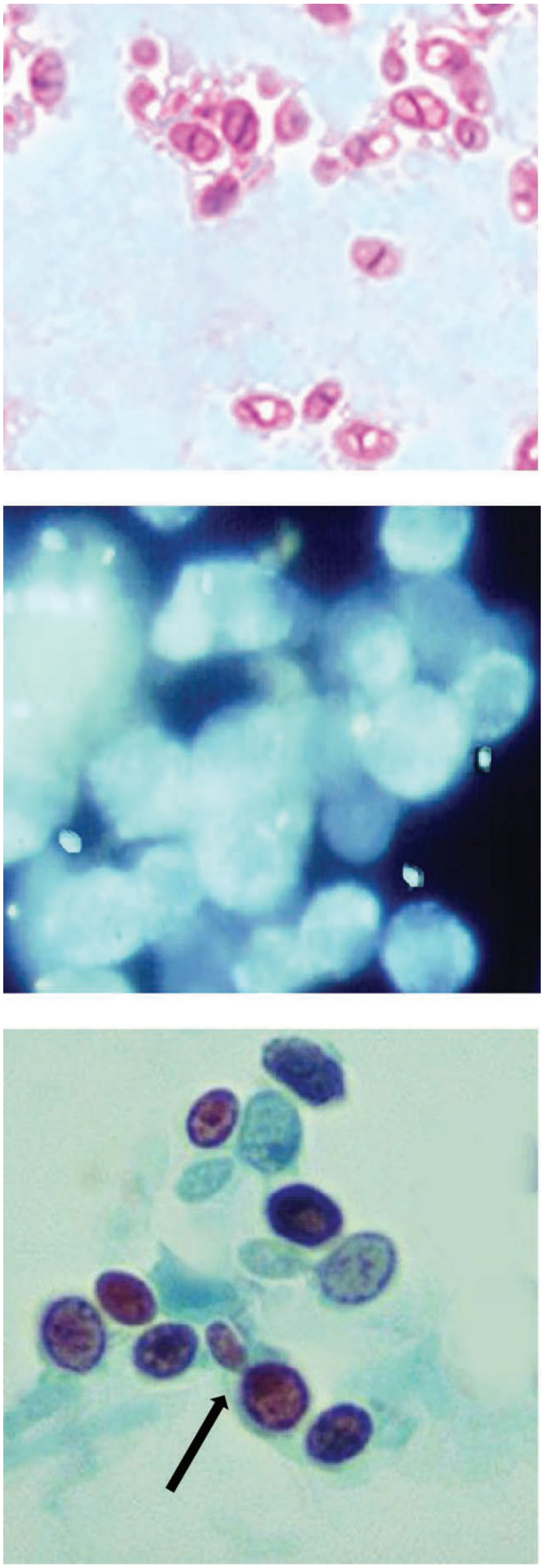


Figure 23.33 Microsporidian spores. (Top) Spores stained with modified trichrome stain (Ryan blue); note the diagonal or horizontal "stripes" that are evidence of the polar tubule. (Middle) Calcofluor white staining of urine sediment; note the small oval intracellular and extracellular spores. This is a nonspecific stain but is more likely to represent a true positive than when seen in a stool specimen containing many fluorescing artifacts. (Bottom) Yeast artifacts; note the large size, lack of the polar tubule, and evidence of budding (arrow).
doi:10.1128/9781555819002.ch23.f33

IgG and IgM antibodies to microsporidia, particularly to *E. cuniculi*. The indirect immunofluorescent-antibody test and the enzyme-linked immunosorbent assay have been the most useful because of the simplicity of the test methods; however, the actual sensitivity and specificity of all the available tests have not yet been defined. Antibodies to *E. cuniculi* and *E. intestinalis* have been found in humans both with and without HIV infection; whether this represents true infection, cross-reactivity with other species, or nonspecific reactions is not clear. Serologic surveys for antibodies to *E. cuniculi* appear to show a link between exposure to a tropical environment and microsporidiosis. Unfortunately, there are no serologic assays available for *E. bieneusi* due to the lack of success in long-term culture and development of available antigens. Whether antibodies to this organism will cross-react with those to *E. cuniculi* is not known. At present, the available serologic data are interesting but the tests are not useful as diagnostic tools for the diagnosis of microsporidiosis.

Although *E. bieneusi* appears to be the most common human microsporidian, serologic evidence is based primarily on results for *E. cuniculi* as the parasite antigen. Whether these surveys reveal actual infections with this organism or whether there is cross-reactivity with other microsporidia is unknown. However, the data are highly suggestive that latent infections with microsporidia occur in a number of groups, ranging from patients with other infections such as tuberculosis, typhoid fever, leprosy, malaria, schistosomiasis, toxoplasmosis, Chagas' disease, and toxocariasis to normal individuals and a group of homosexual men. Microsporidian infections in immunocompetent patients may result in a self-cure following mild symptoms over a short time frame, a situation similar to that seen with both cryptosporidiosis and isosporiasis. As awareness of this infection increases and more sensitive diagnostic techniques are developed, we may find that these infections are not uncommon in immunocompetent hosts.

KEY POINTS—LABORATORY DIAGNOSIS

Microsporidia

1. Approximately 30% of AIDS patients and those with cryptosporidiosis may also have infections with microsporidia. Microsporidian infections must be considered for this group of patients with chronic diarrhea. Identification to genus and species is not possible from light microscopy of stained specimens, including tissues.
2. The modified trichrome staining procedure for stool may be difficult to interpret without positive controls to review. Make sure that the material on the slides is very thin, the smear is stained for the recommended time, and the smear is examined under oil immersion (total magnification of at least ×1,000). When using various stains, optical brightening agents, or experimental immunoassay reagents where diagnosis is based on seeing the actual spores, it is recommended that the fecal specimen be concentrated and centrifuged at 500 × *g* for 10 min. Personal experience in our laboratory has demonstrated a larger number of spores in the concentrated sediment than in the unconcentrated specimen *or in specimens that have been centrifuged at a lower speed or for a shorter time*.
3. The optical brightening agents (calcofluor, Fungi-Fluor, Uvitex 2B) provide a sensitive screening method, but the results are nonspecific. False-positive results have been reported because of fluorescent artifact material.
4. As immunoassay reagents become commercially available, we may see diagnostic procedures with greater specificity and sensitivity. Reagents are currently being used in a few institutions; these preparations are generally easier to read than are the routine stains.
5. Touch preparations can be methanol fixed and stained with Giemsa.
6. Plastic-embedded tissues stained with PAS, silver, acid-fast, and routine hematoxylin-eosin stains generally stain better than paraffin-embedded tissues. This finding may be related to the use of formalin as a tissue fixative. Tissue Gram stains (Brown Brenn, Brown-Hopps) are also highly recommended.
7. Be sure to gain some experience in examining these preparations before sending out patient specimen results. This work almost mandates the use of positive control material, regardless of which technique(s) is used. Once a laboratory has gained experience with these methods, it is appropriate to begin accepting clinical specimens.
8. EM is the "gold standard" (very specific) for confirming infection and for attempting to classify the organisms seen in tissues. However, EM procedures may not be as sensitive as some of the other available methods, due to the very small specimens obtained and examined.
9. It is recommended that more than one procedure be used when working with stool specimens (modified trichrome, optical brightening agent); this is primarily because of the large number of artifacts present in stool. Urine and other body fluid specimens are much easier to examine for the presence of microsporidial spores, since less artifact material is seen in these specimens than in stool.

Treatment

Although a number of drugs have been tried, results have been variable (33). Agents such as metronidazole, itraconazole, octreotide, primaquine, Lomotil, sulfasalazine, loperamide, and albendazole have been used in various patients. In some cases, the diarrhea subsided; however, biopsy specimens showed the continued presence of organisms, which were probably *E. bieneusi*. Over the past few years, confirmatory evidence indicates that a complete parasitological cure is possible with albendazole. However, these patients had disseminated infections with *Encephalitozoon* (*Septata*) *intestinalis*; albendazole, a benzimidazole that binds to β-tubulin, appears to be very effective in treating this particular organism (33). Currently, the recommended dose for ocular (*E. cuniculi*, *V. corneae*), intestinal (*E. bieneusi*, *Encephalitozoon* [*Septata*] *intestinalis*), and disseminated (*E. hellem*, *E. cuniculi*, *E. intestinalis*, *Pleistophora* spp., *Trachipleistophora* spp.) infections with albendazole is 400 mg orally twice a day plus fumagillin. *In vitro*, albendazole has activity at concentrations of 0.1 μg/ml against all of the *Encephalitozoonidae*. Although apparently static rather than cidal effects are seen with *E. bieneusi* infections, treatment with albendazole results in reduction of symptoms in as many as 50% of patients infected with this organism. Albendazole as a systemic agent is recommended when the organisms have been confirmed in urine or nasal smears.

Fumagillin (soluble salt Fumidil B) has activity against microsporidia, and solutions applied topically have been used in corneal infections. The effects of this drug are static rather than cidal, and relapses of infection occur when the treatment has been discontinued. In one study, the efficacy of fumagillin was measured by clearance of *E. bieneusi* from stools and intestinal biopsy specimens; four patients who received fumagillin remained free of *E. bieneusi* after a mean follow-up of 10 months. The dosage is 20 mg once a day orally for 14 days. Also see chapter 36.

Itraconazole can also be recommended to treat ocular, nasal, and paranasal sinus infection caused by *E. cuniculi* parasites when albendazole treatment fails. In a patient with sinusitis and keratoconjunctivitis, the symptoms completely resolved with itraconazole treatment (200 mg/day for 8 weeks) after albendazole therapy (400 mg/day for 6 weeks) was unsuccessful.

Epidemiology and Prevention

The sources of human infections are not yet totally defined. From what we currently know, possibilities include human-to-human and animal-to-human transmission (57). Although there is some speculation that insect microsporidia can infect humans, this is still unknown. Many questions relating to reservoir hosts and possible congenital infections are still unanswered. Primary infection can occur by inhalation or ingestion of spores from environmental sources or by zoonotic transmission. There is serologic evidence to indicate widespread occurrence of antibodies to *E. cuniculi* in humans. Recent data indicate that the presence of *Encephalitozoon intestinalis* was confirmed in tertiary sewage effluent, surface water, and groundwater; *Enterocytozoon bieneusi* was confirmed in surface water; and *V. corneae* was confirmed in tertiary effluent. This study represents the first confirmation, to the species level, of human-pathogenic microsporidia in water, indicating that these parasites may be waterborne pathogens.

The presence of infective spores in human clinical specimens suggests that precautions when handling body fluids and personal hygiene measures such as hand washing may be important in preventing primary infections in the health care setting. However, comprehensive guidelines for disease prevention will require more definitive information regarding sources of infection and modes of transmission.

Algae (*Prototheca*)

Although algae are not parasites, it is important to know what they are and the relationship of opportunistic infections with these organisms to the compromised patient. The genus *Prototheca* contains several species, the most prevalent of which is *Prototheca wickerhamii*. These organisms are achlorophyllic algae found in the slime flux of trees and freshwater environments. Cutaneous infections were reported more than 30 years ago, and subsequent reports describe other manifestations including systemic disease. Disseminated *Prototheca* infections tend to occur in immunocompromised patients (solid organ transplants, hematological malignancies) with high mortality. The other species implicated in human infections is *Prototheca zopfii* (15, 64, 65). Susceptibility to infection is not well defined but may involve the inability of neutrophils to effectively kill phagocytosed organisms. Approximately 100 cases have been reported throughout the temperate and tropical areas of all continents. Protothecosis is being reported with increasing frequency; within the United States the majority of cases have been reported from the southeastern states (9).

Life Cycle and Morphology

Prototheca spp. are unicellular organisms that reproduce asexually by internal septation and cleavage; they then rupture, releasing endospores. The sporangium contains 2 to 16 sporangiospores, which develop into mature endosporulating cells. No sexual reproductive stages have been identified. These organisms are thought to be achlorophyllous mutants of green algae in the genus *Chlorella*.

They are ubiquitous in nature and colonize the human skin, fingernails, respiratory tract, and digestive system.

Clinical Disease

The main underlying conditions are solid organ transplantation, hematological malignancies with or without stem cell transplants, alcoholism, diabetes mellitus, chronic glucocorticoid use, peritoneal dialysis, HIV/AIDS, and cancers. The overall incubation time is not known but is generally considered to be about 2 weeks from the time of trauma and possible implantation of infectious organisms. The majority of infections involve the skin and underlying structures, such as the olecranon bursa (15, 64, 65); however, disseminated infection may also occur. Cutaneous or bursal infection may result from accidental inoculation or from trauma, including surgical. One patient developed nasopharyngeal ulceration due to *P. wickerhamii* after prolonged endotracheal intubation. Bursal infection presents with indolent swelling and tenderness. Cutaneous lesions have been described as painless papules, papulonodules, diffuse erythema, plaques (may be verrucous), and eczematoid or ulcerated areas. Immunocompromised patients may have more severe lesions. In addition to disseminated infection, peritonitis has been found in patients undergoing chronic ambulatory peritoneal dialysis and in the blood of patients with central venous access infections. Infections have also been seen in the gallbladder, peritoneum, and liver in a patient presenting with symptoms consistent with sclerosing cholangitis.

One 80-year-old woman receiving 2 mg of dexamethasone daily presented with tenderness in an area of diffuse erythema of the right arm, forearm, and dorsum of the hand together with several 4- to 6-mm flesh-colored papules. Biopsy revealed granulomatous inflammation with many organisms present in the dermis (15, 64, 65). These organisms stained with both PAS and Gomori's methenamine-silver (GMS) stain and were approximately 5 to 15 µm in diameter. They contained multiple internal septa. Another patient presented with recalcitrant ulcerated papules and plaques on both legs.

This infection has also been found in an HIV-positive patient (65). The patient presented with a nodule in the extensor face of the thumb. A routine excision was performed, and histology revealed numerous granulomas, some with central fibrinoid necrosis. Large numbers of prototheca were found in PAS and GMS stains, showing endosporulation with diagnostic morula- or daisy-like sporangia. On the basis of the relative number of polymorphonuclear leukocytes, lymphocytes, and plasma cells present, the cellular response was considered minimal. The organisms were found extracellularly or within macrophages and giant cells. Protothecal meningitis has also been found in an AIDS patient. In general, these cases are seen in patients who are immunocompromised, either from other underlying diseases or from immunosuppressive therapy (64).

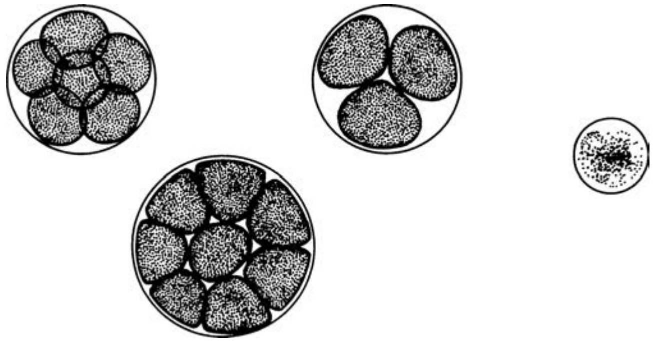
Diagnosis

These algae are easily recovered on routine culture media; however, they are inhibited by cycloheximide, which is present in many selective fungal media. Diagnosis of protothecosis is dependent on histological findings or microbiological isolation of the organism from affected sites or blood. Colony characteristics of *Prototheca* spp. resemble other yeast-like organisms such as *Candida* or *Cryptococcus*. The optimal temperature for growth is 30°C, and these algae grow as white to creamy yeast-like colonies on Sabouraud's medium. Microscopic examination of culture material reveals the same structures as seen in tissue, including spherical sporangia containing multiple sporangiospores (endospores) (Fig. 23.35 to 23.37). The organisms have been described as looking like a spoked wheel. Macroscopically, the cells are variable in size and shape and do not bud or form hyphae. Asexual reproduction occurs through release of sporangiospores from sporangia. Sporangia of *P. zopfii* range from 14 to 16 µm in diameter, and those of *P. wickerhamii* are 7 to 13 µm. Cells of *P. wickerhamii* tend to be round, while those of *P. zopfii* are oval or cylindrical. Both species assimilate glucose and galactose; *P. wickerhamii* also assimilates trehalose. These organisms are nonfermentative.

In cutaneous histologic preparations, the typical tissue response includes a granulomatous inflammation with giant cells, histiocytes, lymphocytes and plasma cells accompanied by the presence of *Prototheca* organisms. The organisms may be numerous or few and can be seen as single cells or clusters. The GMS stain is recommended. In tissue the sporangia are usually larger (30 µm) than those seen in culture (*P. wickerhamii*, 7 to 13 µm; *P. zopfii*, 14 to 16 µm).

Laboratories and clinicians who care for immunocompromised patients should thoroughly investigate "fungemia"

Figure 23.35 Sporangia containing sporangiospores (endospores) of *Prototheca wickerhamii*. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch23.f35



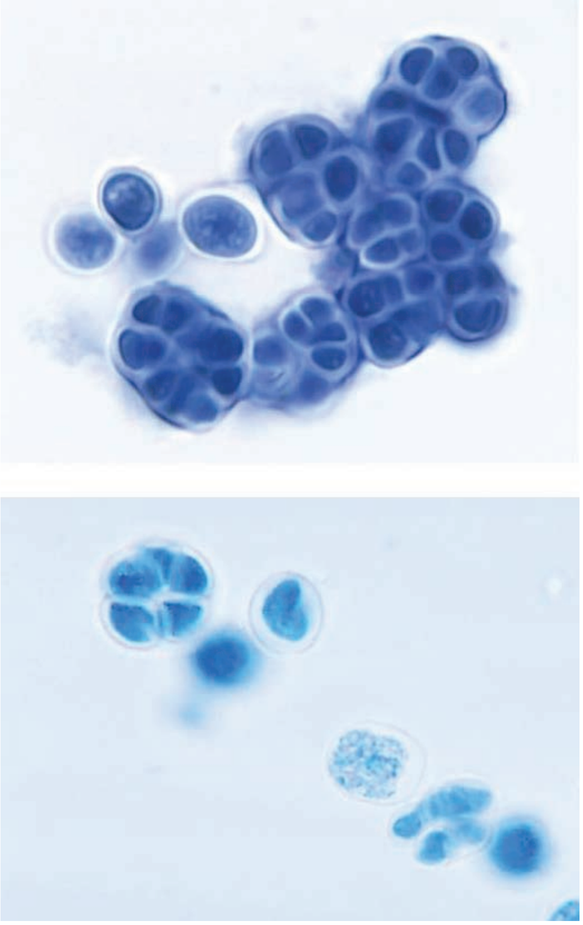


Figure 23.36 Sporangia containing developing sporangiospores (endospores) of *Prototheca* spp. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch23.f36

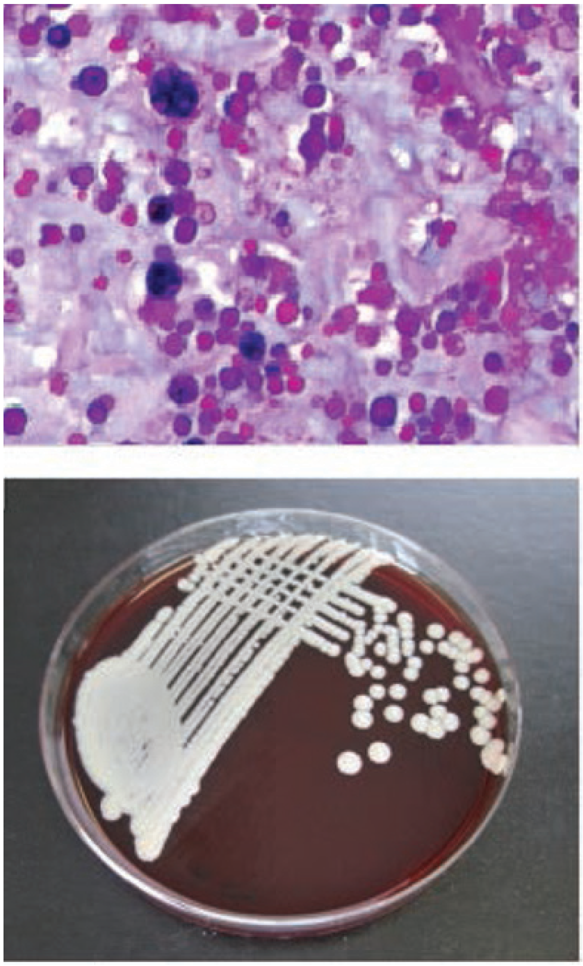


Figure 23.37 *Prototheca wickermanii*. (Upper) Histopathological section of tissue sample obtained from the patient's skin and stained with PAS. Magnification, ×400. (Lower) Isolate on horse blood Columbia agar after incubation at 35°C for 96 h, showing smooth, creamy colonies (courtesy of reference 9). doi:10.1128/9781555819002.ch23.f37

and organism identification, especially in cases of persistent infection that fails to respond to appropriate therapy (9, 38).

Treatment

Most of the protothecal infections are indolent and very slow to heal. Although surgical debridement or excision may be necessary and effective, most infections are treated with antifungal agents. Combined therapy with tetracycline and amphotericin B appears to be effective. Infections that are multifocal or visceral or occur in an immunocompromised host require amphotericin B or combined therapy. Recent reports indicate that itraconazole is effective; however, healing may be gradual. Infections caused by *Prototheca* spp. tend to be resistant to 5-fluorocytosine.

Epidemiology and Prevention

Prototheca spp. have been isolated from a number of different environmental sites, including slime flux of trees, freshwater and seawater, soil, and sewage. Organisms have also been isolated from dogs, cats, cattle, sheep, deer, and some wild animals. *Prototheca* spp. have been recovered from human feces, sputum, skin scrapings, and fingernails, even in cases with no true infectious process. Although infections caused by *Prototheca* spp. are relatively uncommon, the true incidence worldwide may be underestimated. These infections should always be suspected in cases where the skin and subcutaneous tissues are involved and/or olecranon bursitis is present with indolent swelling and tenderness. An awareness of the possibility of infection with these organisms is critical to accurate diagnosis and prompt treatment.

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24

Free-Living Amebae

Naegleria fowleri
Acanthamoeba spp.
Balamuthia mandrillaris
Sappinia (*diploidea*) *pedata*

Infections caused by small, free-living amebae belonging to the genera *Naegleria*, *Acanthamoeba*, *Balamuthia*, and *Sappinia* are less well known or recognized clinically than the intestinal amebae. Also, methods for laboratory diagnosis are unfamiliar and not routinely offered by most laboratories. However, infections caused by these organisms include primary amebic meningoencephalitis (PAM) caused by *Naegleria fowleri;* granulomatous amebic encephalitis (GAE) caused by *Acanthamoeba* spp. and *Balamuthia mandrillaris*; and amebic keratitis, cutaneous lesions, and sinusitis caused by *Acanthamoeba* spp. (Table 24.1) (1–10). Another organism recently identified, *Sappinia* (*diploidea*) *pedata*, has been linked to encephalitis and has been confirmed as a human pathogen. Effective therapy remains a problem with these infections. When free-living, these organisms feed on bacteria and nutrients in moist soil and in freshwater and marine water. The development of diagnostic molecular methods represents a specific and potentially rapid method for the identification of some of these organisms soon after their primary isolation from the environment (1–3).

Although it is well known that both free-living and pathogenic protozoa harbor a variety of endosymbiotic bacteria, it is unclear what role these organisms play in terms of host survival, infectivity, and invasiveness. The study of parasites and symbionts of free-living amebae is relatively new; many of the terms used to describe these relationships are listed in Table 24.2 (6, 11). Serologic evidence that some of the endosymbionts within the free-living amebae might be human pathogens has led to additional studies of these relationships. The ameba-resistant bacteria are varied; some are natural hosts and coexist with free-living amebae, some have been recovered by amebic coculture, and some are resistant to a particular amebic host *in vitro*. Some bacteria are obligate intracellular organisms, others are facultative intracellular bacteria, and some are extracellular. While some of these bacteria are established human pathogens, some are emerging potential pathogens which were formerly considered environmental species. The presence of intracellular bacteria has been detected in axenically grown *Acanthamoeba* isolates. *Legionella pneumophila* can infect, multiply within, and kill both *Naegleria* and *Acanthamoeba* amebae. These amebae may be natural hosts for *Legionella* organisms as well as for other bacteria such as *Campylobacter jejuni*, *Listeria monocytogenes*, *Vibrio cholerae*, *Mycobacterium*

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TABLE 24.1 Free-living amebae causing disease in humans[a]

	Disease parameter					
	Acanthamoeba					
Characteristic	**GAE**	***Acanthamoeba* keratitis**	**Cutaneous lesions, sinusitis**	***Balamuthia* GAE (BAE)**	***Naegleria* PAM**	***Sappinia* Meningitis, encephalitis**
General disease description	Chronic, protracted, slowly progressive CNS infection (may involve lungs); generally occurs in individuals with underlying diseases	Painful, progressive, sight-threatening corneal disease; patients generally immunocompetent	Most common in patients with AIDS, with or without CNS involvement, and in those receiving immunosuppressive therapy for organ transplantation	Chronic, protracted, slowly progressive CNS infection (may involve lungs); generally occurs in individuals with underlying diseases (HIV/AIDS, organ transplantation, steroid therapy), but has been seen in immunocompetent patients	Rare but nearly always fatal infection; migration of amebae to brain through olfactory nerve; symptoms can mimic bacterial meningitis; death usually occurs 3–7 days after onset of symptoms; clinical suspicion based on history is critical	Single case reported in Texas; loss of consciousness, vomiting, photophobia, headache, and blurring of vision; solitary 2-cm mass in the posterior left temporal lobe revealed necrotizing hemorrhagic inflammation containing the trophozoites of free-living amebae
Entry into the body	Olfactory epithelium, respiratory tract, skin, sinuses	Corneal abrasion	Skin, sinuses, respiratory tract	Olfactory epithelium, skin, respiratory tract	Olfactory epithelium	Unknown; environmental organism
Incubation	Weeks to months	Days	Weeks to months	Weeks to months	Days	Unknown
Clinical symptoms	Confusion, headache, stiff neck, irritability	Blurred vision, photophobia, inflammation, corneal ring, pain	Skin lesions, nodules, sinus lesions, sinusitis	Slurred speech, muscle weakness, headache, nausea, seizures	Headache, nausea, vomiting, confusion, fever, stiff neck, seizures, coma	Loss of consciousness, vomiting, photophobia, headache, and blurring of vision
Disease pathology	Focal necrosis, granulomas	Corneal ulceration	Granulomatous reaction in skin, inflammation	Multiple necrotic foci, inflammation, cerebral edema	Hemorrhagic necrosis	Tumor in brain
Diagnostic methods[b]	Brain biopsy, CSF smear/wet prep, culture, indirect immunofluorescence of tissue, PCR	Corneal scrapings or biopsy, stain with calcofluor white, culture, confocal microscopy	Skin lesion biopsy, culture, indirect immunofluorescence of tissue	Brain biopsy, culture on mammalian cells, indirect immunofluorescence of tissue	Brain biopsy, CSF wet prep, culture, indirect immunofluorescence of tissue, PCR	Brain biopsy

[a] Table information adapted from references 4–10.

[b] Indirect immunofluorescence on tissue and PCR methods available from CDC.

TABLE 24.2 Definitions pertaining to the relationships among free-living amebae and other organisms[a]

Description	Definition
Adaptation	Selection pressure = change in an organism
Ameba-resistant microorganisms	Microorganisms that evolved to resist destruction by amebae, neither internalized nor killed while within the ameba
Ameba-resistant bacteria	Bacteria that evolved to resist killing by amebae
Character	Phenotypic characteristics of an organism
Crib	"Baby bed"—free-living amebae that serve as a reservoir and incubator of new antibiotic-resistant bacteria = adaptation to human macrophages
Commensalism	Symbiosis—one organism benefits and the other neither is harmed nor benefits
Endosymbiont	Symbiont that lives within another organism
Endosymbiotic	During portion of life cycle or throughout; no lysis activity
Lytic	Lyses the host cell
Mutualism	Symbiosis in which both organisms benefit
Parasite	Benefits from an association but is harmful to the host
Symbiont	Lives in close contact with another organism during most of life cycle
Symbiosis	Two organisms living in close contact for most of their life spans
Symbiosis island	Gene cluster that confers symbiotic traits, which may be transferred horizontally
Trojan horse	Protozoan "horse" that may bring ameba-resistant organisms into the human host
Virulence	Pathogenicity

[a]Adapted from references 6 and 11.

leprae, *Burkholderia cepacia*, *Burkholderia pickettii*, and *Pseudomonas aeruginosa* (6, 11, 12). Because of their well-known resistance to chlorine, the amebic cysts are considered to be vectors for these intracellular bacteria. This can have tremendous significance for any hospital where the water source is contaminated with free-living amebae. There is also evidence to suggest that the presence of amebae in domestic water supplies may provide growth conditions that enhance the pathogenicity of the organisms for the human host (13). These amebae are also acceptable hosts for *Chlamydia pneumoniae*, echoviruses, and polioviruses (11).

Naegleria fowleri

Culbertson and his colleagues in 1958 were the first to develop the concept that free-living soil and water amebae could cause disease in humans. A number of fatal cases of acute meningoencephalitis have been reported since that time (8, 9). Almost all patients whose cases were documented in the years immediately following this awareness of amebic infections had a history of swimming in freshwater lakes or ponds a few days prior to the onset of symptoms.

Infections of the central nervous system (CNS) caused by free-living amebae have been recognized only since the mid-1960s, and our understanding of this disease process is still incomplete. One type of meningoencephalitis (PAM) is a fulminant and rapidly fatal disease that affects mainly children and young adults. The disease closely resembles bacterial meningitis but is caused by *Naegleria fowleri*, an organism found in moist soil and freshwater habitats. Close to 200 cases of PAM have occurred worldwide, and approximately 90 of those cases have been reported from the United States. Until recently, it was thought that this infection was limited to humans; however, infections have also been reported in other animals.

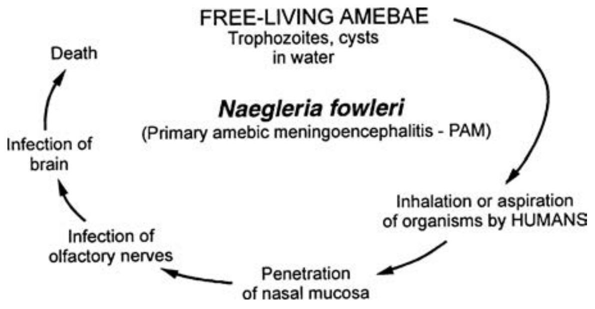
Life Cycle and Morphology

N. fowleri has a three-stage life cycle: trophozoite, flagellate, and cyst, with the stage primarily depending on environmental conditions (Fig. 24.1). Trophozoites can be found in water or moist soil and can be maintained in tissue culture or other artificial media.

Morphology of Trophozoites. The trophozoites can occur in two forms, ameboid and flagellate (Fig. 24.2 through 24.4). Motility can be observed in hanging-drop preparations from cultures of cerebrospinal fluid (CSF); the ameboid form (the only form recognized in humans) is elongate with a broad anterior end and tapered posterior end (Table 24.3). The size ranges from 7 to 35 µm. The diameter of the rounded forms is usually 10 to 18 µm. There is a large, central karyosome and no peripheral nuclear chromatin. The cytoplasm is somewhat granular and contains vacuoles (Fig. 24.2).

Morphology of Flagellates. The ameboid-form organisms change to the transient, pear-shaped flagellate form when they are transferred from culture or teased from tissue into

Figure 24.1 Life cycle of *Naegleria fowleri*. doi:10.1128/9781555819002.ch24.f1


FREE-LIVING AMEBAE
Trophozoites, cysts in water
Naegleria fowleri
(Primary amebic meningoencephalitis - PAM)
Inhalation or aspiration of organisms by HUMANS
Penetration of nasal mucosa
Infection of olfactory nerves
Infection of brain
Death

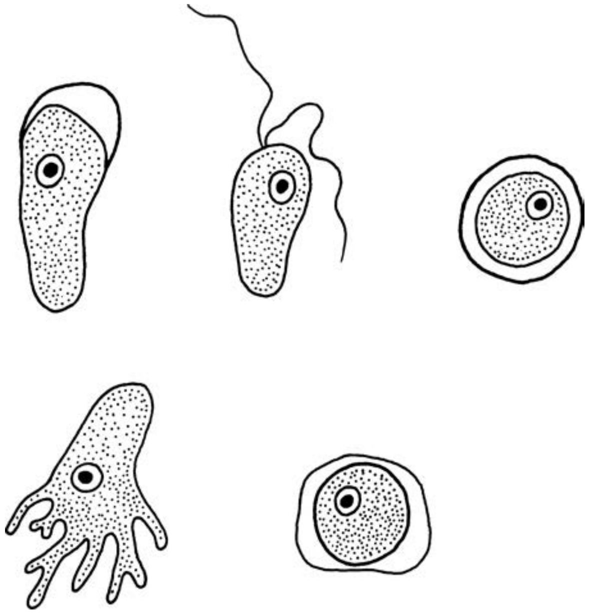


Figure 24.2 Diagram of trophozoite. (Upper row) Flagellate and cyst forms of *Naegleria fowleri*; (lower row) trophozoite and cyst of *Acanthamoeba* spp. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch24.f2

water and maintained at a temperature of 27 to 37°C. The change may occur very quickly (within a few hours) or may take as long as 20 h. The flagellate form has two flagella at the broad end. Motility is typical, with either spinning or jerky movements. These flagellate forms do not divide, but when the flagella are lost, the ameboid forms resume reproduction.

Morphology of Cysts. Cysts from nature and from agar cultures look the same and have a single nucleus almost identical to that seen in the trophozoite. They are generally round, measuring from 7 to 15 μm, and there is a thick double wall (Fig. 24.2; Table 24.3). They are not seen in tissues.

Clinical Disease

PAM caused by *N. fowleri* is an acute, suppurative infection of the brain and meninges (Fig. 24.5). With extremely rare exceptions, the disease is rapidly fatal in humans. The period between contact with the organism and onset of clinical symptoms such as fever, headache, and rhinitis may vary from 2 to 3 days to as long as 7 to 15 days.

The amebae may enter the nasal cavity by inhalation or aspiration of water, dust, or aerosols containing the trophozoites or cysts. The organisms then penetrate the nasal mucosa, probably through phagocytosis of the olfactory epithelium cells, and migrate via the olfactory nerves to the brain (4, 8). Data suggest that *N. fowleri* directly ingests brain tissue by producing food cups or amebostomes, in addition to producing a contact-dependent cytolysis which is mediated by a heat-stable hemolytic protein, heat-labile cytolysis, and/or phospholipase enzymes (8). Cysts of *N. fowleri* are generally not seen in brain tissue.

Early symptoms include vague upper respiratory distress, severe bifrontal or bitemporal headache, lethargy, photophobia, fever, and occasionally olfactory problems. The acute phase includes sore throat, stuffy blocked or discharging nose, and severe headache. Progressive symptoms include spiking fever of 40°C, vomiting, and stiffness of the neck. Mental confusion and coma usually occur approximately 3 to 5 days prior to death. The cause of death is usually cardiorespiratory arrest and pulmonary edema. PAM usually occurs in children and young adults who have recently swum in lakes, heated swimming pools with inadequate chlorination, or other bodies of freshwater that contain amebae.

There are no defined differences between PAM and acute bacterial meningoencephalitis. The peripheral white blood count is usually elevated, with a predominance of neutrophils. CSF may be clear or appear grayish to yellowish-white and may be tinged with red. CSF pressure is usually elevated, ranging from 300 to 600 mm H_2O. CSF pleocytosis is usually seen, with a predominance of neutrophils, a condition which may be mistaken for bacterial infection. Early in the infection the CSF white cell count may be low, but during disease progression it may increase to 26,000 cells/mm^3. The CSF glucose level is usually low to normal and CSF protein is high, ranging from 100 to 1,000 mg/dl. Thus, PAM and acute purulent bacterial meningitis may be difficult to differentiate, particularly in the early stages. Unfortunately, if the CSF Gram stain is interpreted incorrectly (identification of bacteria as a false positive), the resulting antibacterial therapy has no impact on the amebae and the patient will usually die within several days.

Extensive tissue damage occurs along the path of amebic invasion; the nasopharyngeal mucosa shows ulceration, and the olfactory nerves are inflamed and necrotic. Hemorrhagic necrosis is concentrated in the region of the olfactory bulbs and the base of the brain with pockets of amebic trophozoites within edematous and hemorrhagic CNS tissue. Organisms can be found in the meninges, perivascular spaces, and sanguinopurulent exudates.

The first case of organ transplantation from a donor who had died of undiagnosed *N. fowleri* infection was reported in 1997 (8). While no subsequent amebic infections occurred in the three organ recipients, this report emphasizes the need for adequate evaluation of the benefits and risks of transplanting tissues from persons whose illness might have been caused by an infectious agent. Apparently, the Centers for Disease Control and Prevention (CDC) is also aware of another instance in which kidneys

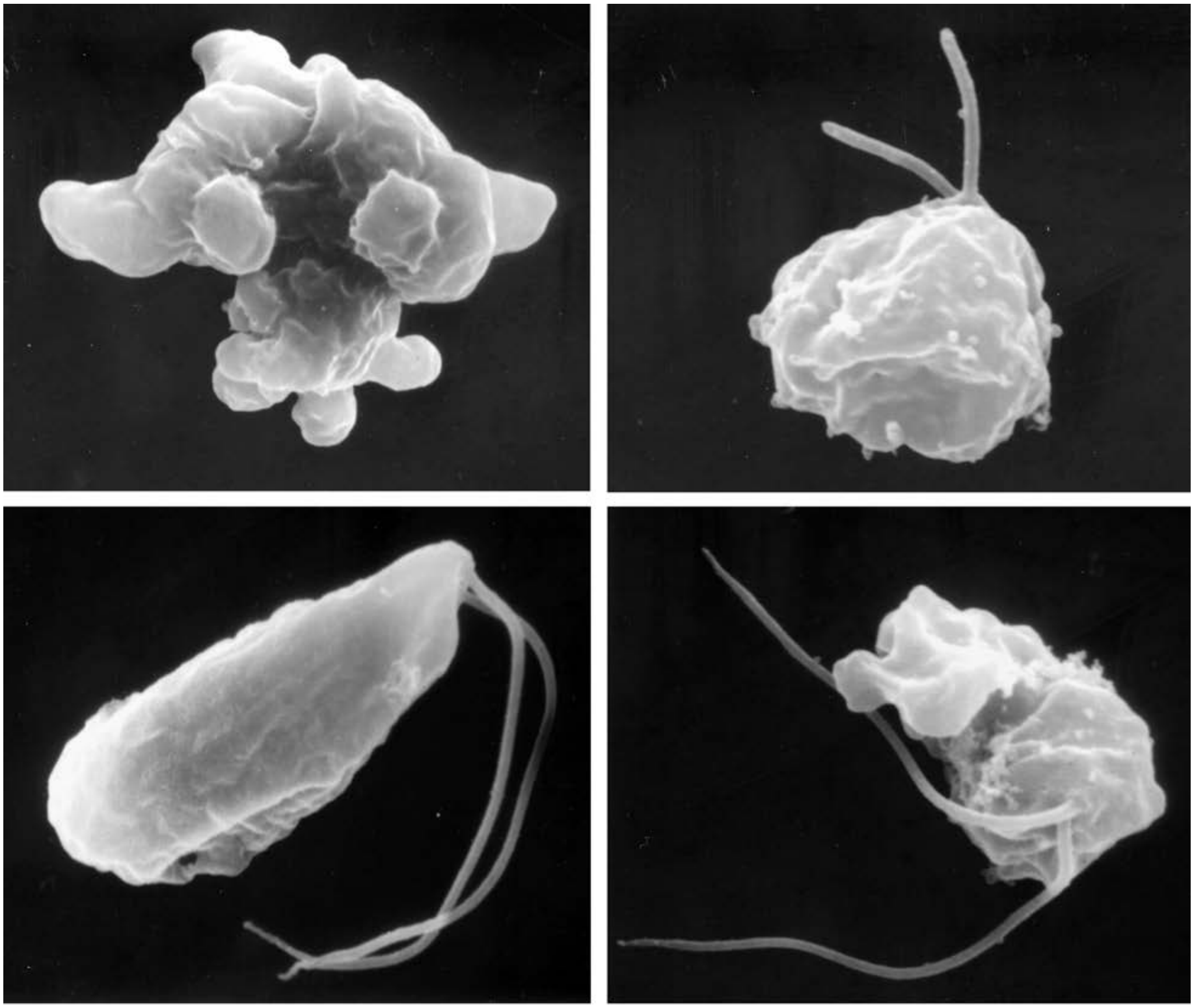


Figure 24.3 (Upper, left) Scanning electronic micrograph (SEM) of *N. fowleri* (Lovell strain) ameboid cell with numerous blunt pseudopods; (right) SEM of *N. fowleri* (Lovell strain) preflagellate, rounded and producing two short, blunt flagella. (Lower, left) SEM of *N. fowleri* (Lovell strain) flagellate with two long, pointed flagella emerging from the anterior rostrum; (right) SEM of *N. fowleri* (Lovell strain) reverting flagellate with two long, pointed flagella. (Courtesy of D. T. John, reprinted from Cable BL, John DT, *J Protozool* **33:**467–472, 1986, with permission.) doi:10.1128/9781555819002.ch24.f3

were transplanted prior to determination that the donor was infected with *N. fowleri* (8). At present, the risk of transmission of *N. fowleri* by donor organs has not been clarified, and no practical test is available to ensure that donor organs are organism free. Also, no prophylactic drug regimen to treat transplant recipients has been established.

Although most PAM infections are associated with recreational freshwater exposure, recent reports indicate that ablution, including nasal rinsing, has been associated with *N. fowleri* cases globally (14). In the United States, during 2003–2012, 3 of 31 persons infected with *N. fowleri* became infected after performing nasal rinsing with contaminated tap water. Two of the three patients performed nasal rinsing using a neti pot or similar device (14, 15) (Fig. 24.6). Detection of *N. fowleri* in the shower and hot water heater suggests that the organism had colonized the home's plumbing system and points to the home as the likely site of exposure. It is imperative that patients understand the need for using safe water to reduce the risk of becoming infected with *Naegleria* during ritual nasal rinsing and ablution (Fig. 24.7) (Table 24.4).

Diagnosis

Clinical and laboratory data usually cannot be used to differentiate pyogenic meningitis from PAM, and so the diagnosis may have to be reached by a process of elimina-

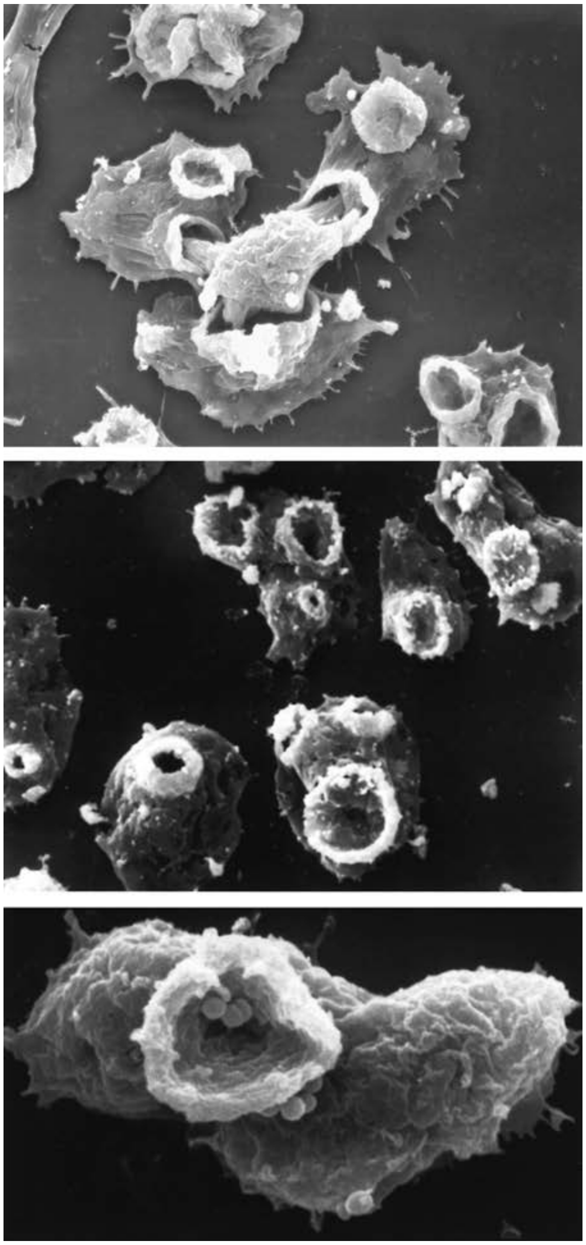


Figure 24.4 (Top) Three amebae of the pathogenic free-living ameba *N. fowleri* attacking and engulfing a fourth, presumably dead, ameba. The amebae are using sucker-like structures (amebostomes) in this novel form of phagocytosis. (Courtesy of D. T. John, from John DT, Cole TB Jr, Carciano-Cabral FM, *Appl Environ Microbiol* **47**:12–14, 1984). (Middle) *N. fowleri* trophozoites. (Bottom) *N. fowleri* trophozoite ingesting starch granules. (Middle and bottom images courtesy of F. M. Marciano-Cabral.) doi:10.1128/9781555819002.ch24.f4

TABLE 24.3 Comparison of *Naegleria fowleri* and *Acanthamoeba* spp.

Characteristic	*Naegleria fowleri*	*Acanthamoeba* spp.
Trophozoite	Biphasic (amebic) and flagellate forms; 8–15 (7–35) µm; lobate pseudopodia (amebic form)	Large (15–25 µm): no flagella; filiform pseudopodia
Cysts	Not present in tissue; small, smooth, rounded; 7–15 µm	Present in tissue; large with wrinkled double wall
Growth on media	Require living cells (bacteria or cell culture); do not grow with >0.4% NaCl	May grow without bacteria; not affected by 0.85% NaCl
Appearance in tissue[a]	Smaller than *Acanthamoeba* spp.; dense endoplasm; less distinct nuclear staining	Large; rounded; less endoplasm; nucleus more distinct

[a] *Entamoeba histolytica* has a delicate nuclear membrane and a small, pale-staining nucleolus. Freshwater amebae have a distinct nuclear membrane and a large, deep-staining nucleolus.

tion. A high index of suspicion is often mandatory for early diagnosis (8). Although most cases are associated with exposure to contaminated water through swimming or bathing, this is not always the case. The rapidly fatal course of 3 to 6 days after the beginning of symptoms (with an incubation period of 1 day to 2 weeks) requires early diagnosis and immediate chemotherapy if the patient is to survive.

Analysis of the CSF shows decreased glucose and increased protein concentrations. Leukocytes may range from several hundred to >20,000 cells/mm^3. Gram stains and bacterial cultures of CSF are negative; however, the Gram stain background debris can incorrectly be identified as bacteria, thus leading to incorrect therapy for the patient.

A definite diagnosis could be made by demonstration of the amebae in the CSF or in biopsy specimens. Either CSF or sedimented CSF (500 × *g* for 10 min) should be placed on a slide, under a coverslip, and observed for motile trophozoites; smears can also be stained with Wright's or Giemsa stain. Some have recommended a lower centrifugation speed (250 × *g*); however, routine centrifugation does not appear to be problematic. CSF, exudate, or tissue fragments can be examined by light microscopy or phase-contrast microscopy. Although phase-contrast is recommended, bright-field illumination with reduced light is acceptable. Care must be taken not to mistake leukocytes for actual organisms or vice versa. It is very easy to confuse leukocytes and amebae, particularly when one is examining CSF by using a counting chamber, hence the recommendation to use just a regular slide and coverslip. Motility may vary, and so the main differential characteristic is the spherical nucleus with a large karyosome.

After all but 0.5 ml of the supernatant has been aspirated, the sediment should be inoculated onto the center of an agar plate that has been seeded with bacteria; the plate should then be incubated at 37°C. Biopsy or autopsy material should be triturated in a small amount of ameba saline (0.5 ml), placed in the center of a nonnutrient agar plate seeded with *Escherichia coli* bacteria as a food source, and incubated in the same way (16, 17) (Fig. 24.8). Under a microscope, *N. fowleri* amebae appear as small, motile blotches. After 2 to 3 days of incubation, the amebae begin to encyst. After 4 to 5 days of incubation, trophozoites and cysts should be visible.

Specimens should never be refrigerated prior to examination. Use of smears stained with Giemsa or Wright's stain or a Giemsa-Wright's stain combination can also be helpful. If *N. fowleri* is the causative agent, trophozoites only are normally seen. If the infecting organism is *Acanthamoeba*, cysts may also be seen in specimens from patients with CNS infection. Unfortunately, most cases are diagnosed at autopsy; confirmation of these tissue findings must include culture and/or special staining with monoclonal reagents in indirect fluorescent-antibody procedures (Fig. 24.9).

In cases of presumptive pyogenic meningitis in which no bacteria are identified in the CSF, the computed tomography appearance of basal arachnoiditis (obliteration of basal cisterns in the precontrast scan with marked enhancement after the administration of intravenous contrast medium) should alert the staff to the possibility of acute PAM.

The amebae can be identified in histologic preparations by indirect immunofluorescence and immunoperoxidase techniques. The organism in tissue sections looks very much like an *Iodamoeba bütschlii* trophozoite, with a very large karyosome and no peripheral nuclear chromatin; the organisms can also be seen with routine histologic stains (Fig. 24.5).

In infections possibly caused by *Acanthamoeba* spp., periodic acid-Schiff (PAS) stains the cyst wall red and methenamine silver stains the cyst black; these stains may be helpful. Normally, *Naegleria* and *Acanthamoeba* isolates are identified to the species level by a reference laboratory, such as the CDC, using indirect fluorescent-antibody procedures with a monoclonal or polyclonal antibody. The formalin stability of at least one of the epitopes may be useful in detecting *N. fowleri* in fixed biopsy specimens and in investigating the pathologic process (8).

A multiplex real-time PCR is available at CDC that identifies all three amebae (*Acanthamoeba* sp., *B. mandrillaris*, and *N. fowleri*). The real-time multiplex PCR is a rapid, sensitive, and specific assay, which can be performed and results reported within 4 to 5 hours.

In general, serologic tests have not been helpful in the diagnosis of this infection. The disease progresses so rapidly that the patient is unable to mount an immune response.

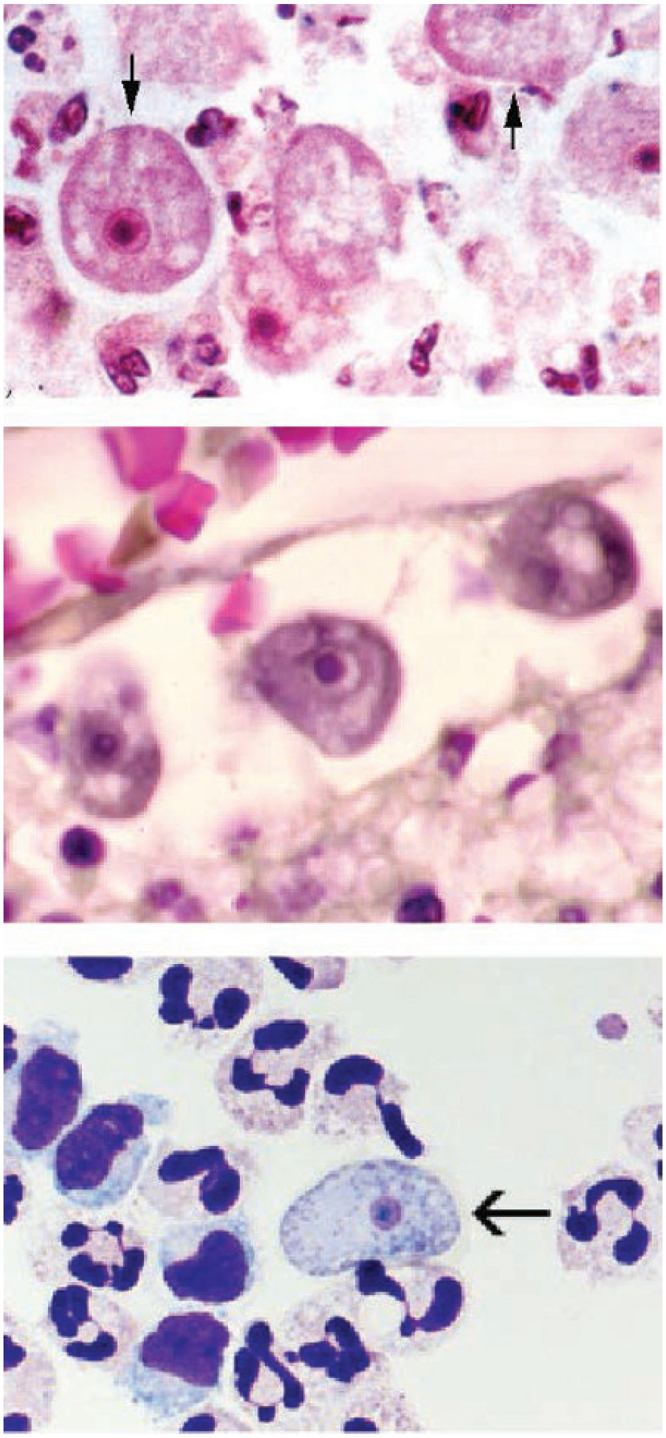


Figure 24.5 *Naegleria fowleri.* (Top, middle) Organisms in brain tissue from a human with PAM. (Bottom) A cytospin of fixed CSF showing a *Naegleria fowleri* trophozoite stained with Giemsa-Wright stain (courtesy of the CDC Public Health Image Library).
doi:10.1128/9781555819002.ch24.f5

KEY POINTS—LABORATORY DIAGNOSIS

Naegleria fowleri

1. Never refrigerate the specimen(s) prior to examination or culture.
2. Beware of the false-positive Gram stain, especially since PAM usually mimics bacterial meningitis and debris may be confused with bacteria (CSF contains increased numbers of leukocytes, increased protein concentrations, and decreased sugar concentrations).
3. The *Naegleria* trophozoites mimic leukocytes in a counting chamber. Motility is more likely to be seen if a drop of CSF is placed directly on a slide and a coverslip is added. Phase-contrast optics are recommended; however, if regular bright-field microscopy is used, the light level should be low. The slide can also be warmed to 35°C to stimulate trophozoite motility.
4. Organisms can be cultured on nonnutrient agar plated with *Escherichia coli*.
5. Low-speed centrifugation is recommended for CSF (150 × *g* for 5 min); however, routine centrifugation speed and time (500 × g for 10 min) also appear to be successful.
6. Incubate the plate in room air at 35 to 37°C for tissues from the CNS and at 30°C for tissues from other sites. *If* a single agar plate is used with no particular causative agent suspected, 30°C is acceptable and will also allow recovery of *Naegleria*. If *Naegleria* is strongly suspected (acute CNS infection), culture at 35 to 37°C for maximum growth.
7. Using the low-power objective (10×), observe the plates daily for 7 days.
8. Remember that *B. mandrillaris* does not grow on agar plates seeded with bacteria.
9. Standard precautions should be used when handling these specimens and cultures. Procedures should be performed in a biological safety cabinet.

Treatment

Many antimicrobial and antiparasitic drugs have been screened for *in vitro* and *in vivo* activity against *N. fowleri*. Although *N. fowleri* is very sensitive to amphotericin B *in vitro*, only a few patients have recovered after receiving intrathecal and intravenous injections of this drug alone or in combination with miconazole (8, 18). One case within California in which the patient was successfully treated with intrathecal and intravenous amphotericin B, miconazole, and oral rifampin has been documented (19). There

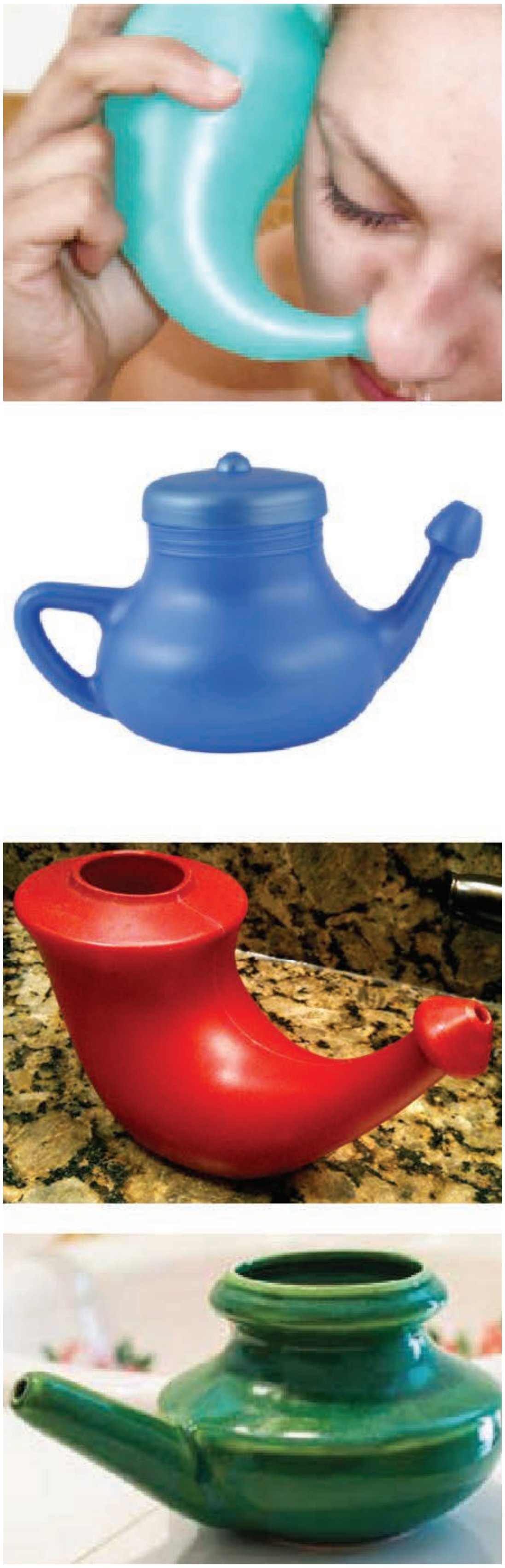


Figure 24.6 Examples of neti pots for nasal irrigation.
doi:10.1128/9781555819002.ch24.f6

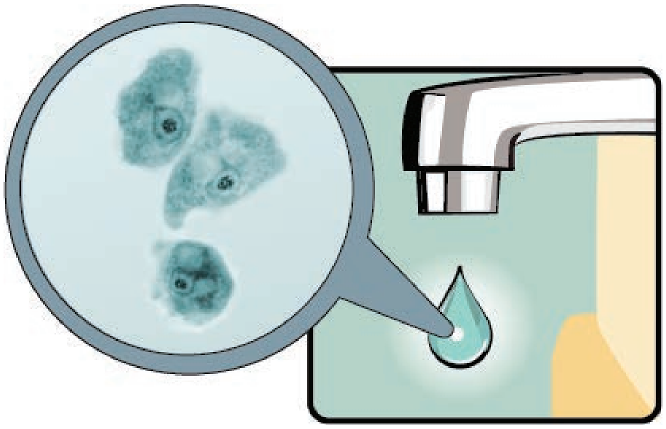


Figure 24.7 *Naegleria fowleri*: warning sign indicating possible contamination of tap water with pathogenic protozoa (courtesy of the CDC Public Health Image Library).
doi:10.1128/9781555819002.ch24.f7

TABLE 24.4 Safe water for ritual nasal rinsing and ablution (prevention of *Naegleria* infection)[a]

Method or type of water	Comments
Boil	Use water that has been previously boiled for 1 minute and left to cool. At elevations above 6,500 feet, boil for 3 minutes.
Filter	Use a filter designed to remove some water-loving microbes. The label may read "NSF 53" or "NSF 58." Filter labels that read "absolute pore size of 1 micron [1 µm] or smaller" are also effective.
Buy	Use water with a label specifying that it contains distilled or sterile water.
Disinfect: clear water[b]	Add 1/8 teaspoon (or 8 drops; about 0.625 ml) of unscented liquid household chlorine (5–6%) bleach for each gallon of clear water (or 2 drops of bleach for each liter or each quart of water), Stir the mixture well. Let it stand for 30 minutes or longer before you use it. Store the disinfected water in clean, sanitized containers with tight covers.
Disinfect: cloudy water[b]	Filter it through a clean cloth, paper towel, or coffee filter OR allow any sediment to settle. Draw off the clear water. Add 1/8 teaspoon (or 8 drops; about 0.625 ml) of unscented liquid household chlorine (5–6%) bleach for each gallon of clear water (or 2 drops of bleach for each liter or each quart of water), Stir the mixture well. Let it stand for 30 minutes or longer before you use it. Store the disinfected water in clean, sanitized containers with tight covers.

[a] Adapted from CDC information related to ritual nasal rinsing and ablution.

[b] *Naegleria fowleri* does not survive in sea water and has not been detected in sea water.

was a synergistic effect with miconazole and amphotericin B *in vitro*; however, rifampin was ineffective. Dexamethasone and phenytoin were also given to this patient to decrease intracranial pressure and seizure, respectively. *Naegleria* infections have also been treated successfully with amphotericin B, rifampin, and chloramphenicol; amphotericin B, oral rifampin, and oral ketoconazole; and amphotericin B alone (8, 9, 18). Delay in diagnosis and the fulminant nature of PAM result in few survivors; this is unfortunate since *N. fowleri* is quite sensitive to the antifungal agent amphotericin B.

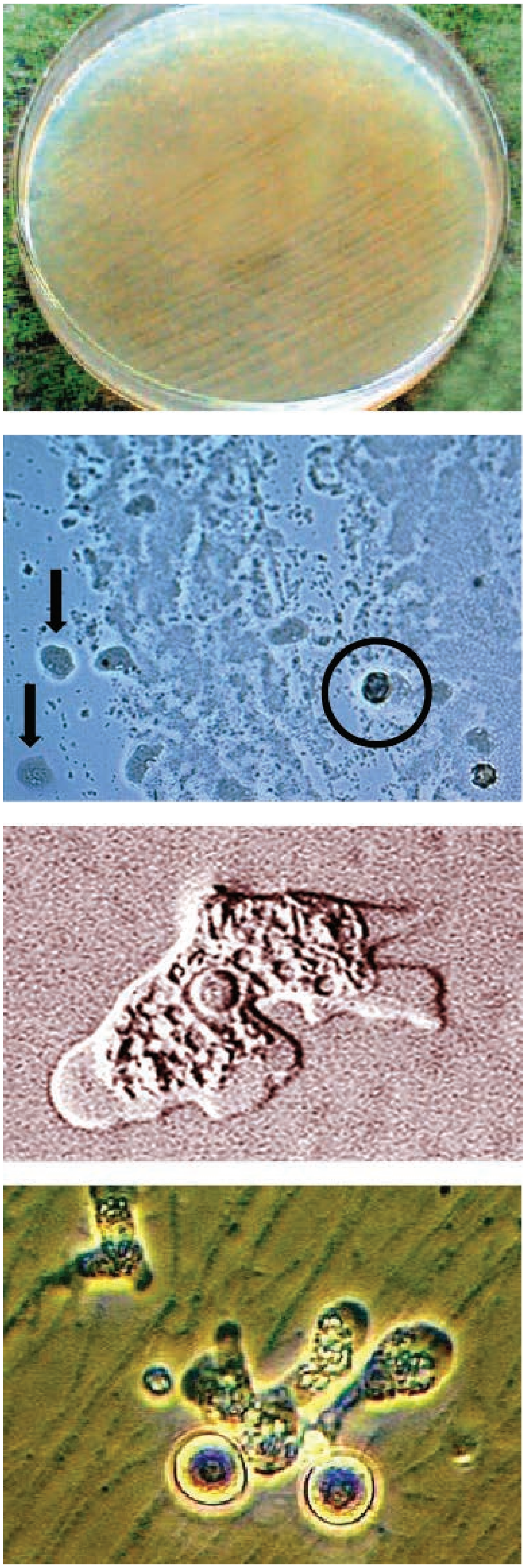
Epidemiology and Prevention

Nearly 200 presumptive or proven cases of PAM have been reported in the literature, including cases from the United States, Ireland, England, Belgium, Czechoslovakia, Australia, New Zealand, Brazil, and Zambia. Clinical patient histories indicate exposure to the organism via freshwater lakes or swimming pools shortly before onset; patients had been previously healthy with no specific underlying problems (8). Pathogenic *Naegleria* organisms have also been isolated from nasal passages of individuals with no history of water exposure, suggesting the possibility of airborne exposure.

The first isolations of the environmental strains of pathogenic *N. fowleri* were reported from water and soil in Australia and from sewage sludge samples in India. Detection of *N. fowleri* in heated discharge water has been reported in Belgium and Poland. Since then, there have been additional reports describing isolations of virulent or avirulent strains of *N. fowleri* from the environment. Studies in Belgium have clearly indicated that *N. fowleri* strains are present in artificially heated waters (power plant warm discharge), and studies in the United States have indicated that virulent strains are also found in lakes in geographic latitudes with water temperatures of 14 to 35°C that are totally isolated from any source of thermal discharge (8). These data suggest that the presence of pathogenic or potentially pathogenic amebic strains may depend on both climate and modification of the natural environment. The ability of the cysts to survive under various environmental conditions has been investigated by several workers. These findings suggest that *N. fowleri* cysts produced in the warm summer months may survive the winter and are capable of growth during the following summer.

General preventive measures include public awareness of potential hazards of contaminated water. It has been recommended that warm discharge water not be used for sports and recreational purposes (8), particularly since DNA restriction fragment profiles of environmental strains and human isolates were homogeneous.

In one study, 30 strains of *N. fowleri* were investigated by the randomly amplified polymorphic DNA (RAPD)



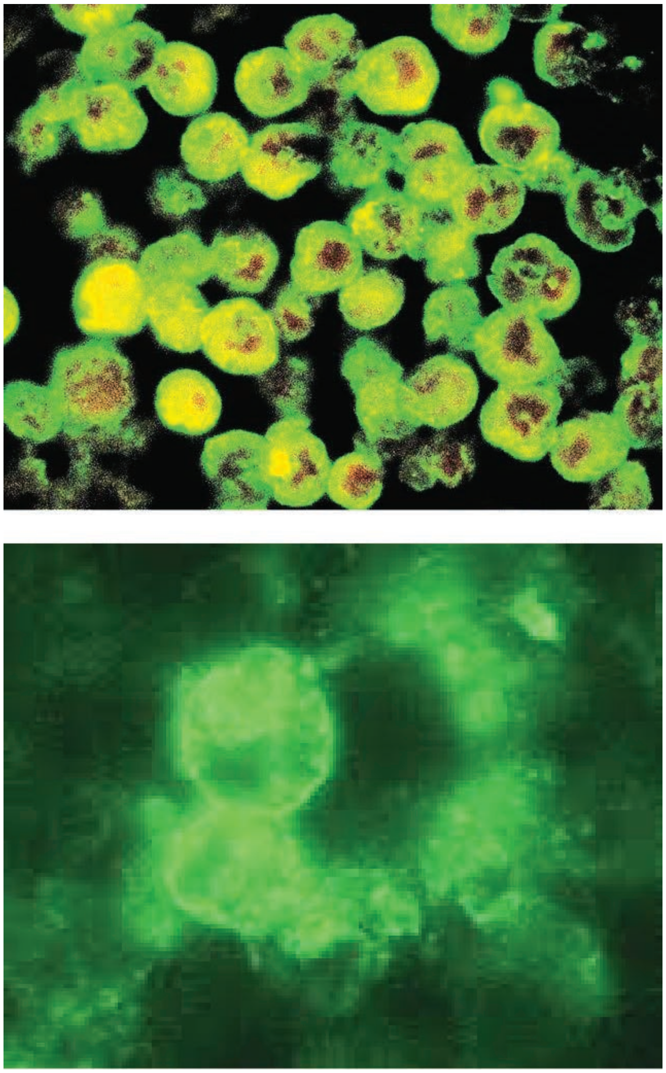


Figure 24.9 *Naegleria fowleri*. (Upper) Direct fluorescent antibody stain; (lower) indirect immunofluorescence (IIF) assay for *Naegleria fowleri*, 1,000× oil magnification (courtesy of the CDC Public Health Image Library).
doi:10.1128/9781555819002.ch24.f9

Figure 24.8 *Naegleria fowleri*. (Top) Nonnutrient agar plate seeded with a lawn of *Escherichia coli*, a food source for free-living amebae; *Naegleria* are growing on the bacterial lawn (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). (Row 2) Trophozoites (arrows) and cysts (circle) on the agar surface culture. (Row 3) Trophozoite from culture in wet mount. (Bottom) Trophozoite ingesting bacteria on surface of agar (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch24.f8

method. The data confirmed earlier work indicating that RAPD variation is not correlated with geographic origin. In particular, Mexican strains belong to the variant previously detected in Asia, Europe, and the United States. In France, strains gave RAPD patterns identical to those of the Japanese strains. All of these strains, in addition to another strain from France, exhibited similarities to South Pacific strains. The results confirmed the presence of many variants in Europe, while only two variants were detected in the United States; these two variants were different from the South Pacific variants (8).

Acanthamoeba spp.

Formerly, the genus *Acanthamoeba* was included in the genus *Hartmanella*. In 1975, Sawyer and Griffin proposed a new family, *Acanthamoebidae*, for the spiny amebae, with *Acanthamoeba* as the type genus and *Acanthamoeba castellanii* as the type species (4). The most characteristic feature of this genus is the presence of spine-like pseudopods called acanthapodia. Several species of *Acanthamoeba* (*A. culbertsoni*, *A. castellanii*, *A. polyphaga*, *A. astronyxis*, *A. healyi*, and *A. divionensis*) cause GAE, primarily in immunosuppressed, chronically ill, or otherwise debilitated persons. These patients tend to have no relevant history involving exposure to recreational freshwater. *Acanthamoeba* spp. also cause amebic keratitis, and it is estimated that to date approximately 1,000 cases of *Acanthamoeba* keratitis have been seen in the United States. Species of *Acanthamoeba* linked to keratitis include *A. castellanii*, *A. polyphaga*, *A. hatchetti*, *A. culbertsoni*, *A. rhysodes*, *A. griffini*, *A. quina*, and *A. lugdunensis* (6). The number of cases worldwide has also been increasing; apparently, the incidence of *Acanthamoeba* keratitis in the United Kingdom is 15 times that in the United States and 7 times that in Holland (20).

The pathogenesis of *Acanthamoeba* infection involves the ability of the organism to invade tissues. This capability depends on adherence to mucosal surfaces, migration through tissues, and the release of oxygen radicals and proteases that can destroy connective tissue. Studies have found that alkaline cysteine proteinases were more active in pathogenic strains whereas serine proteases were found in both pathogenic and nonpathogenic strains (6).

Humoral immunity and complement activation appear to be the primary defense mechanisms against *Acanthamoeba* infections; serum antibodies are mainly the IgM and IgG classes. Both antibody and complement promote recognition of the organisms by phagocytic cells. The amebae are destroyed by neutrophils, which are activated by lymphokines to accelerate the respiratory burst and to release lysosomal enzymes. Macrophages and neutrophils are the major inflammatory cell types found in tissues surrounding amebae. Although the cornea is infiltrated by neutrophils and there is a measurable humoral response, the disease will progress to corneal opacity or perforation without therapy in the immunocompetent patient. It has been suggested that the organisms mask their antigens from cellular immune responses, thus suppressing macrophage functions and/or preventing neutrophil activity. Total serum immunoglobulins do not differ significantly between healthy subjects and patients with *Acanthamoeba* keratitis, but levels of ameba-specific tear IgA were significantly lower in patients with keratitis (6). Although *Acanthamoeba* infections stimulate a granulomatous response, the response in AIDS patients is minimal or absent, findings which are consistent with the poor immune response in these patients.

Another type of meningoencephalitis, GAE, caused by freshwater amebae is less well defined and may occur as a subacute or chronic disease with focal granulomatous lesions in the brain (Table 24.5). The route of CNS invasion is thought to be hematogenous, with the primary site being the skin or lungs. In this infection, both trophozoites and cysts can be found in the CNS lesions. An acute-onset case of fever, headache, and pain in the neck preceded by 2 days of lethargy has also been documented (6). The causative organisms are probably *Acanthamoeba* spp. in most cases,

TABLE 24.5 *Acanthamoeba* infection: clinical manifestations of patients with AIDS[a]

General	Neurologic	Nasal	Cutaneous	Musculoskeletal
Fever	Headache	Congestion	Papule	Abscess
Chills	Mental changes	Discharge	Lump	
Fatigue	Seizures	Tenderness	Disseminated nodules	
Weight loss	Motor deficit	Ulcer	Foot ulcer	
	Cranial nerve involvement	Hemorrhage	Abscesses	
	Meningeal signs	Chronic sinusitis		
	Sensory deficits			

[a] Adapted from Sison JP et al, *Clin Infect Dis* **20:**1207–1216, 1995.

but it is possible that other genera are involved (6, 8). In the acute-onset case mentioned above, *A. culbertsoni* was repeatedly found in and cultured from the CSF. The patient responded dramatically to a combination therapy of penicillin and chloramphenicol. Cases of GAE have been reported in chronically ill or immunologically impaired hosts; however, some patients apparently have no definite predisposing factor or immunodeficiency. Conditions associated with GAE include malignancies, systemic lupus erythematosus, HIV infection, Hodgkin's disease, skin ulcers, liver disease, pneumonitis, diabetes mellitus, renal failure, rhinitis, pharyngitis, and tuberculosis. Predisposing factors include alcoholism, drug abuse, steroid treatment, pregnancy, systemic lupus erythematosus, hematologic disorders, AIDS, cancer chemotherapy, radiation therapy, and organ transplantation. This infection has become more widely recognized in AIDS patients, particularly those with a low CD4$^+$ cell count.

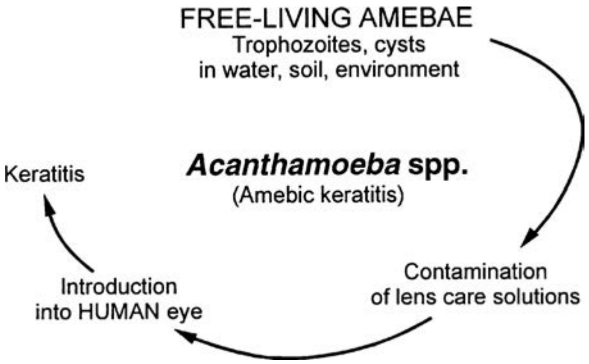
The *Acanthamoeba* group also causes keratitis and corneal ulceration. There are a number of published cases that emphasize the need for clinicians to consider acanthamoebic infection in the differential diagnosis of eye infections that fail to respond to bacterial, fungal, or viral therapy. These infections are often due to direct exposure of the eyes to contaminated materials or solutions. It is now recognized that the wearing of contact lenses is the leading risk factor for keratitis. Conditions which are linked with disease include the use of homemade saline solutions, poor contact lens hygiene, and corneal abrasions.

Life Cycle and Morphology

Unlike *N. fowleri*, *Acanthamoeba* spp. do not have a flagellate stage in the life cycle, only the trophozoite and cyst (Fig. 24.10 and 24.11) (Table 24.3).

Morphology of Trophozoites. Motile organisms have spine-like pseudopods; however, progressive movement is usually not very evident (Fig. 24.2, 24.12, and 24.13). There is a wide range (25 to 40 µm), with the average diameter of the trophozoites being 30 µm. The nucleus has a typical large karyosome, like that seen in *N. fowleri*. This morphology can be seen on a wet preparation. In the environment, these organisms feed on bacteria, algae, and yeast; however, they can also exist on liquid nutrients taken

Figure 24.10 Life cycle of *Acanthamoeba*, the etiologic agent of amebic keratitis. doi:10.1128/9781555819002.ch24.f10


FREE-LIVING AMEBAE
Trophozoites, cysts
in water, soil, environment
Acanthamoeba spp.
(Amebic keratitis)
Contamination
of lens care solutions
Introduction
into HUMAN eye
Keratitis

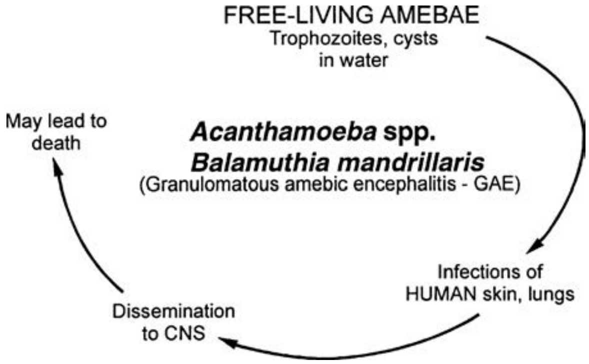
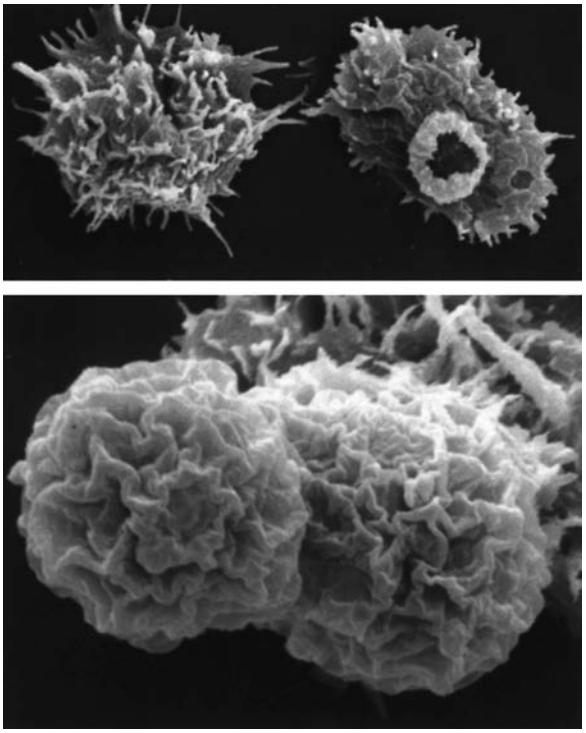

FREE-LIVING AMEBAE
Trophozoites, cysts
in water
Acanthamoeba spp.
Balamuthia mandrillaris
(Granulomatous amebic encephalitis - GAE)
Infections of
HUMAN skin, lungs
Dissemination
to CNS
May lead to
death

Figure 24.11 Life cycle of *Acanthamoeba* and *Balamuthia mandrillaris*, the etiologic agents of GAE. doi:10.1128/9781555819002.ch24.f11

Figure 24.12 (Upper) *Acanthamoeba* trophozoites (note the spiky acanthapodia). (Lower) *Acanthamoeba* cysts. (Images courtesy of F. M. Marciano-Cabral.) doi:10.1128/9781555819002.ch24.f12



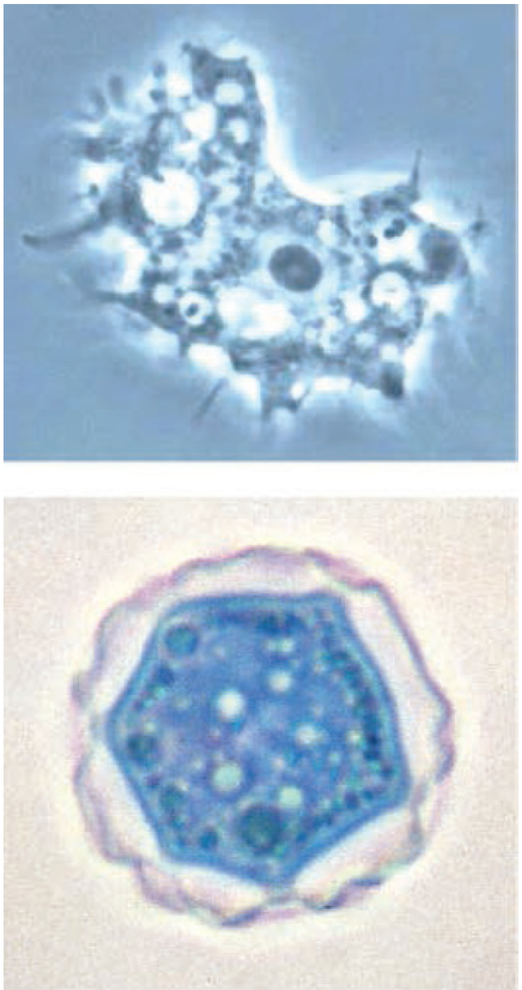


Figure 24.13 (Upper) *Acanthamoeba* trophozoite (note the spiky acanthapodia). (Lower) *Acanthamoeba* cyst (note the double-walled cyst). doi:10.1128/9781555819002.ch24.f13

up through pinocytosis (6). Organelles typically found in higher eukaryotic cells have been identified and include a Golgi complex, smooth and rough endoplasmic reticula, free ribosomes, digestive vacuoles, mitochondria, and microtubules.

Morphology of Cysts. The cysts are usually round with a single nucleus, also having the large karyosome seen in the trophozoite nucleus. The double wall is usually visible, with the slightly wrinkled outer cyst wall and what has been described as a polyhedral inner cyst wall (6) (Fig. 24.2, 24.12, and 24.13). Cysts are uninucleate and have a proteinaceous ectocyst and an endocyst made of cellulose, thus making the cyst visible using calcofluor white. This cyst morphology can be seen in organisms cultured on agar plates. Cyst formation occurs under adverse environmental conditions; cysts are resistant to biocides, chlorination, and antibiotics and can also survive low temperatures (0 to 2°C).

Clinical Diseases

Granulomatous amebic encephalitis. *Acanthamoeba* sp. GAE tends to affect immunosuppressed, chronically ill, or otherwise debilitated persons, generally with no particular history of exposure to freshwater sources. There have been a few reported infections in immunocompetent individuals with no known immunodeficiency or risk factors. *Balamuthia mandrillaris* GAE tends to affect the very young or very old and patients with HIV/AIDS. Whether caused by *Acanthamoeba* sp. or *B. mandrillaris*, GAE is a slowly progressive disease of the CNS.

Meningoencephalitis caused by *Acanthamoeba* spp. may present as an acute suppurative inflammation of the brain and meninges like that seen with *N. fowleri* infection. However, *Acanthamoeba* spp. are generally reported to cause a more chronic, protracted, slowly progressive form of meningoencephalitis. Due to the confusion in the earlier literature regarding the nomenclature of *Acanthamoeba* and *Hartmanella* spp., these organisms have been referred to in the past as belonging to the *Hartmanella-Acanthamoeba* group. However, since no true *Hartmanella* species has been found to be pathogenic to humans, references to *Hartmanella* in human tissues should be revised to read *Acanthamoeba* or *Balamuthia* spp. (6, 21).

The incubation period of GAE is unknown; several weeks or months are probably necessary to establish the disease. The clinical course tends to be subacute or chronic and is usually associated with trauma or underlying disease, not swimming. GAE is characterized by confusion, dizziness, drowsiness, nausea, vomiting, headache, lethargy, stiff neck, seizures, and sometimes hemiparesis. Within the CNS, the cerebral hemispheres are the most likely tissue to be involved. There may be edema and hemorrhagic necrosis within the temporal, parietal, and occipital lobes. A chronic inflammatory exudate can be seen over the cortex, mainly of polymorphonuclear leukocytes and mononuclear cells. Unlike in PAM caused by *N. fowleri*, both trophozoites and cysts are found throughout the tissue. Granulomatous inflammation necrosis and thrombosed vessels are seen in the brain (Fig. 24.14). Multinucleated giant cells forming granulomas can be seen in immunocompetent patients, while granuloma formation is generally poor or lacking in immunocompromised individuals. It is unknown whether brain necrosis is due to direct tissue destruction caused by the organisms, to inflammatory cytokines such as interleukin-1 or tumor necrosis factor, or both. Also, dissemination to other tissues such as the liver, kidneys, trachea, and adrenals can occur in immunocompromised individuals; more unusual sites also include the ears and necrotic bone from a bone graft of the mandible (6).

In the differential diagnosis, other space-occupying lesions of the CNS (tumor, abscess, fungal infection, etc.) must also be considered. Some of the predisposing factors have included Hodgkin's disease, diabetes, alcoholism, pregnancy, and steroid therapy. Organisms have also been found in the adrenal gland, brain, eyes, kidneys, liver, pancreas, skin, spleen, thyroid gland, and uterus.

Amebic meningoencephalitis can also be an unusual complication of bone marrow transplantation. A case has been reported in a patient with non-Hodgkin's lymphoma after autologous stem cell transplantation. Leg weakness, fever, and urinary retention developed 69 days after transplantation. The patient then developed fever, seizures, rapid deterioration of mental functions, and hypercapneic respiratory failure. Magnetic resonance imaging demonstrated a ring-enhancing lesion at the level of thoracic spines 11 and 12. Pleocytosis was seen in the CSF. On autopsy, a subacute meningoencephalitis secondary to *A. culbertsoni* infection was seen (6).

AIDS Patients. The first case of an *Acanthamoeba* infection in a patient with AIDS was in 1986, and many additional cases have been reported since that time. *Acanthamoeba* spp. are now well accepted as opportunistic pathogens in immunocompromised patients with AIDS. Unfortunately, most of the cases have resulted in death and have been confirmed postmortem. These individuals usually have advanced HIV disease with a very low $CD4^+$ T-cell count, generally less than 200/mm^3. Often the patients expire within a month after the onset of CNS symptoms. Unfortunately, the diagnosis of this infection requires a high index of suspicion, since both clinical and histologic findings may mimic those of disseminated fungal or algal disease (Tables 24.5 and 24.6). The primary site of infection is thought to be the sinuses and lungs. The skin is also thought to be a possible portal of entry. Clinical manifestations of patients with AIDS and *Acanthamoeba* infection can be reviewed in Table 24.5. They may include general complaints such as fever and chills, nasal congestion or lesions, neurologic symptoms, and musculoskeletal and cutaneous lesions. Some patients, especially those with AIDS, can develop erythematous nodules, chronic ulcerative skin lesions, or abscesses. Although CSF lymphocytosis is seen in non-AIDS patients, the CSF may reveal no cells in HIV-positive patients. These patients also tend to exhibit chronic sinusitis, otitis, and cutaneous lesions. Patients with CNS symptoms and AIDS may be misdiagnosed with toxoplasmosis, although serologic tests for *Toxoplasma* may be negative. Other patients have been misdiagnosed with CNS vasculitis, squamous cell carcinoma, or bacterial meningitis.

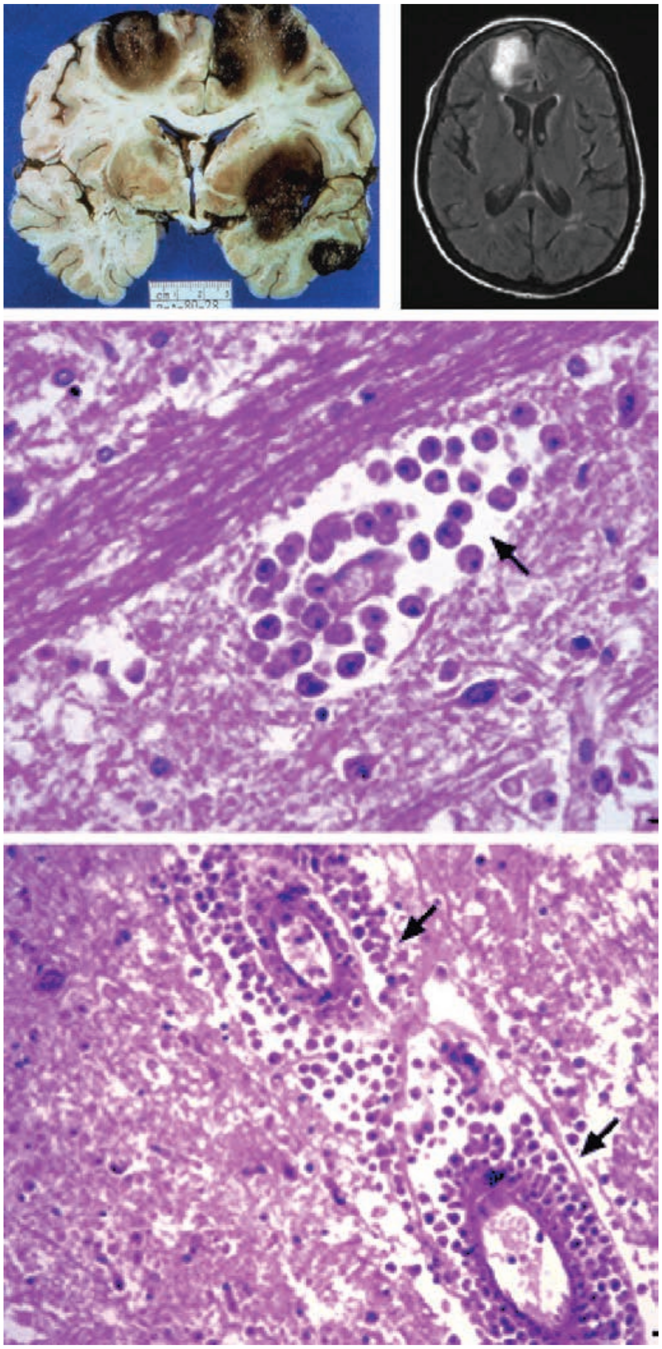


Figure 24.14 *Acanthamoeba.* (Top, left) Coronal section of the cerebral hemispheres with cortical and subcortical necrosis from a fatal human case of GAE; (right) magnetic resonance image of the patient's brain after the first seizure, showing a hemorrhagic lesion in the right frontal lobe. (Middle) Hematoxylin and eosin stain of brain tissue from a human with GAE. Numerous trophozoites can be identified within the tissue (arrow). (Bottom) Trophozoites identified within vascular walls (arrow). All images courtesy of A. J. Martinez.
doi:10.1128/9781555819002.ch24.f14

TABLE 24.6 *Acanthamoeba* infection: examples of clinical features in specific patients with AIDS[a]

Other illnesses[b]	Initial diagnosis	Clinical syndrome
Chronic sinusitis, PCP, disseminated MAC, CMV retinitis	Disseminated fungal infection	Suspected GAE, disseminated cutaneous infection
PCP, esophageal candidiasis, recurrent sinusitis	Cat scratch disease or vasculitis	Disseminated cutaneous infection
Toxoplasma encephalitis, CMV retinitis, giardiasis	Kaposi's sarcoma	Nasal, disseminated cutaneous infection, possible GAE
Cryptococcal meningitis, oral candidiasis, disseminated herpes zoster	*Toxoplasma* encephalitis or lymphoma	GAE
PCP, MAC infection, Kaposi's sarcoma	*Acanthamoeba* infection	Nasal ulcer (single nodule), intramuscular abscess
Inflammatory demyelinating polyneuropathy, esophageal candidiasis, varicella zoster, dendritic keratitis	*Acanthamoeba* infection	Disseminated cutaneous infection, possible GAE

[a] Adapted from Sison JP et al, *Clin Infect Dis* **20**:1207–1216, 1995.
[b] CMV, cytomegalovirus; MAC, *Mycobacterium avium* complex; PCP, *Pneumocystis* pneumonia.

Both PAM and GAE carry a grave prognosis and, although considered rare, should be considered in the differential diagnosis of patients who present with appropriate histories and imaging findings, including nonspecific brain edema on computed tomography in PAM and focal punctate enhancing lesions in the posterior cranial fossa on T1-weighted magnetic resonance imaging in GAE. The initial symptoms are commonly fever and headache. It is important to remember that in patients thought to have toxoplasmosis, review of the cranial computed tomogram could not distinguish between the two etiologic agents *Toxoplasma* and *Acanthamoeba*.

In patients with disseminated disease, the duration of infection from onset to death can range from 7 days to 5 months. Once neurologic symptoms become obvious, the survival time is generally about a month. Since early therapy can alter the clinical outcome, recognition of this disease as a potential cause of the patient's presentation is critical. It is important to initiate appropriate diagnostic testing for confirmation of the causative agent as soon as possible.

Keratitis. Keratitis, uveitis, and corneal ulceration have been associated with *Acanthamoeba* spp. Infections have been seen in both hard- and soft-lens wearers, and particular attention has been paid to soft-lens disinfection systems, including homemade saline solutions. Heat disinfection overall appears to be more effective than cold disinfection methods in killing *Acanthamoeba* trophozoites and cysts. Recent studies indicate that not all commercial contact lens disinfectant solutions are equal in their ability to kill *A. castellanii* (22). Solutions containing hydrogen peroxide or chlorhexidine-thimerosal were active against both trophozoites and cysts. A benzalkonium chloride-based solution was effective only against trophozoites, and solutions containing polyaminopropyl biguanide or polyquaternium 1 were completely ineffective. Unfortunately, a lack of standard methods for testing disinfection solutions may also complicate the issue, although the use of a more reproducible approach may be helpful (22). Obviously, the cyst form of the parasite is much more resistant, and the need to test any solution for both trophozoites and cysts is critical. In addition to the active ingredient(s), it is important to consider adequate exposure times. In general, products containing hydrogen peroxide were the most active, demonstrating the best minimum trophozoite amebicidal concentration for both trophozoites (3 min) and cysts (9 h). It is always important to remember that different products containing hydrogen peroxide at the same concentration may have different killing properties; one report indicated that when hydrogen peroxide was used according to the manufacturer's directions (30 min of contact time), it was not effective against *A. castellanii* trophozoites and cysts. An excellent discussion of the manufacturer's minimum recommended disinfection time for contact lens solutions can be found in reference 4. In order for a disinfectant to be cysticidal, it must be able to gain access to the trophozoite contained within the cyst wall. The most likely route would be the pores in the double cell wall; however, it is thought that these pores (ostioles) are plugged with mucopolysaccharide. Thus, the disinfectant would need to be able to disrupt these "plugs" in order to reach the trophozoite.

The onset of corneal infection with *Acanthamoeba* spp. can vary tremendously; however, two factors often appear to be involved: trauma and contaminated water. When corneal abrasions occur, the disease process is usually more rapid, with ulceration, corneal infiltration, iritis, scleritis, severe pain, hypopyon (pus in the anterior chamber), and loss of vision. When this process occurs in an individual who wears contact lenses, the onset is more gradual but the results are often the same. A contact lens can act as a mechanical vector for transport of amebae present in the storage case onto the cornea. Subsequent multiplication and invasion of the tissue may occur. Another consideration involves the potential infection of the nasal cavity via lacrimal drainage, a condition that probably causes no problems in a healthy individual. However, it remains unknown whether the presence of these organisms in the nasopharynx of an immunocompromised individual increases the risk of GAE.

Decreased corneal sensation has contributed to the misdiagnosis of *Acanthamoeba* keratitis as herpes simplex keratitis; this mistake can also be attributed to the presence of irregular epithelial lesions, stromal infiltrative keratitis, and edema, which are commonly seen in herpes simplex keratitis (Fig. 24.15). *Acanthamoeba* keratitis may be present as a secondary or opportunistic infection in patients with herpes simplex keratitis. Unfortunately, as a result, treatment can be delayed for 2 weeks to 3 months. The presence of nonhealing corneal ulcers and the presence of ring infiltrates are also clinical signs that alert the ophthalmologist to the possibility of amebic infection.

It is important to consider *Acanthamoeba* infection in the differential diagnosis of uveitis in patients with AIDS. It is also important to remember that incisional keratotomy may predispose the cornea to delayed-onset infectious keratitis; *Acanthamoeba* should always be considered as a possible cause of infection, and clinical specimens should be cultured in refractory cases.

One of the mechanisms of *Acanthamoeba* adhesion to the corneal surface may involve interactions between the mannose-binding protein of *Acanthamoeba* and mannose-containing glycoproteins on the surface of the corneal epithelium (6, 8). It appears that enhanced growth is seen in surface-attached amebae and that cocontamination of lens systems with bacteria may be a prime factor in the development of amebic keratitis. Data also suggest that obligate bacterial endosymbionts are able to enhance the amebic pathogenic potential *in vitro*, although the exact mechanisms have not yet been identified (11). There are also data to show that monoclonal anti-*Acanthamoeba* IgA antibodies can protect against *Acanthamoeba* keratitis, suggesting that this occurs by inhibiting adhesion of the amebae to the corneal epithelium (6).

Acanthamoeba spp. produce a variety of proteases, which may facilitate cytolysis of the corneal epithelium, invasion of the extracellular matrix, and dissolution of the corneal stromal matrix (8). *Acanthamoeba* spp. are also resistant to complement lysis. The ability of *Acanthamoeba* spp. to activate the alternative complement pathway but to resist complement-mediated cellular lysis can be attributed to both the release of a transport-dependent extracellular matrix and the presence of complement-inhibitory surface proteins.

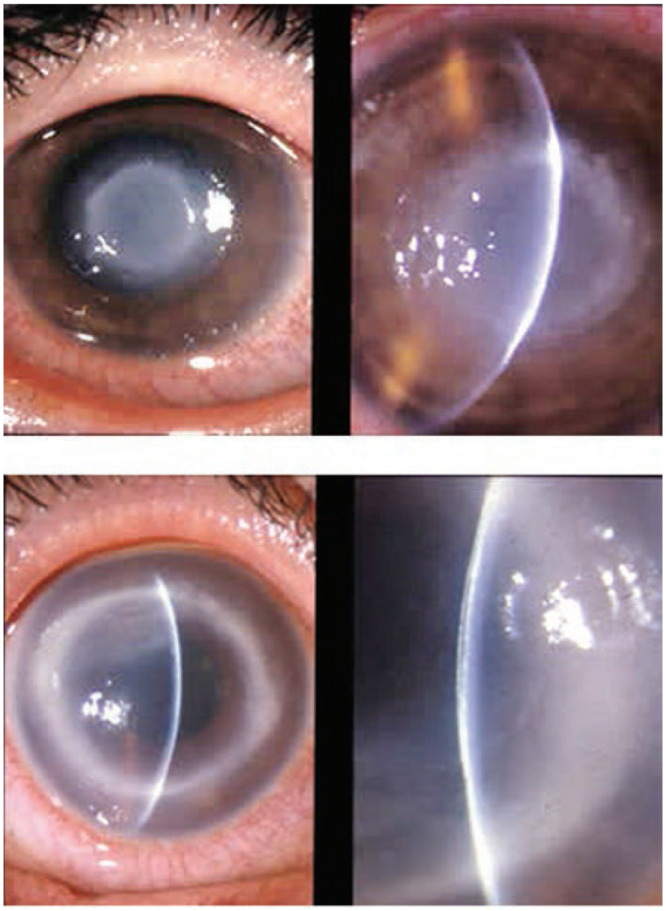


Figure 24.15 *Acanthamoeba* keratitis. (Upper) Left, broad illumination; right, slit beam illumination. Early, central stromal inflammation (keratitis); resembles the immunogenic form of herpes simplex stromal keratitis. (Lower) Left, broad illumination with slit beam; right, high magnified slit beam. Typical advanced ring infiltrate. (Courtesy of the CDC Public Health Image Library, photographs by Dan B. Jones.)
doi:10.1128/9781555819002.ch24.f15

Although previous studies have shown that early diagnosis and therapy are definitely related to outcome, differences among patients with and without contact lenses should also be considered. The mean time to diagnosis could be significantly longer in patients who are not contact lens users than in those who are, primarily due to the differences in the index of suspicion regarding the link between contact lens use and *Acanthamoeba* keratitis. These differences could be directly related to poor outcome, with patients who did not wear contact lenses having a very poor visual acuity or penetrating keratoplasty compared with patients who wore contact lenses. It is important to remember that all patients with unresponsive microbial keratitis, even those who do not wear contact lenses, should be evaluated for possible *Acanthamoeba* infection.

Cutaneous Lesions. Although cutaneous infections are more common in patients with AIDS, regardless of the presence or absence of CNS involvement, they have also been seen in immunocompetent patients. The disease includes the presence of hard erythematous nodules or skin ulcers (Fig. 24.16 and 24.17). The early lesions appear as firm papulonodules that drain purulent material; these lesions then develop into nonhealing indurated ulcers. Although disseminated skin lesions may be the first sign of *Acanthamoeba* infection, it is unclear whether the skin lesions represent a primary focus or may result from hematogenous spread from other body sites. Although the mortality rate for these individuals without CNS involvement is around 75%, it increases to 100% if cutaneous infection is accompanied by CNS disease (6).

Histology reveals necrosis surrounded by inflammatory cells, vasculitis, and the presence of trophozoites and cysts of the amebae (Fig. 24.18). However, these findings can also mimic skin lesions caused by fungi, viruses, mycobacteria, or foreign-body reactions. The amebae can also resemble yeast forms of *Blastomyces dermatitidis*, sporangia of *Rhinosporidium seeberi*, *Cryptococcus neoformans*, or *Prototheca wickerhamii*. Because cutaneous acanthamoebiasis has been misdiagnosed as a number of different conditions, it is important to repeat the examination of biopsy material with a different specimen if the first results are negative and no amebae are seen.

Diagnosis

Nonnutrient Agar Plate Culture. Laboratory examinations similar to those for *N. fowleri* can be used to recover and identify these organisms; the one exception is recovery by culture, which has not proven to be as effective with GAE patients infected with *B. mandrillaris*. The most effective approach uses nonnutrient agar plates with Page's saline and an overlay growth of *Escherichia coli* on which the amebae feed (6, 8) (Fig. 24.19). There is also evidence that phosphate-buffered saline can be used. Specimens transported in ameba-saline (5.0 ml) and filtered through 13-mm, 0.22-µm-pore-size cellulose acetate and nitrate filters (Millipore Corp., Bedford, MA) have also been acceptable for organism recovery. The filter is then placed in the center of the nonnutrient agar plate seeded with *E. coli*. Tissue stains are also effective, and cysts can be stained with Gomori's silver methenamine, PAS, and calcofluor white (6, 8).

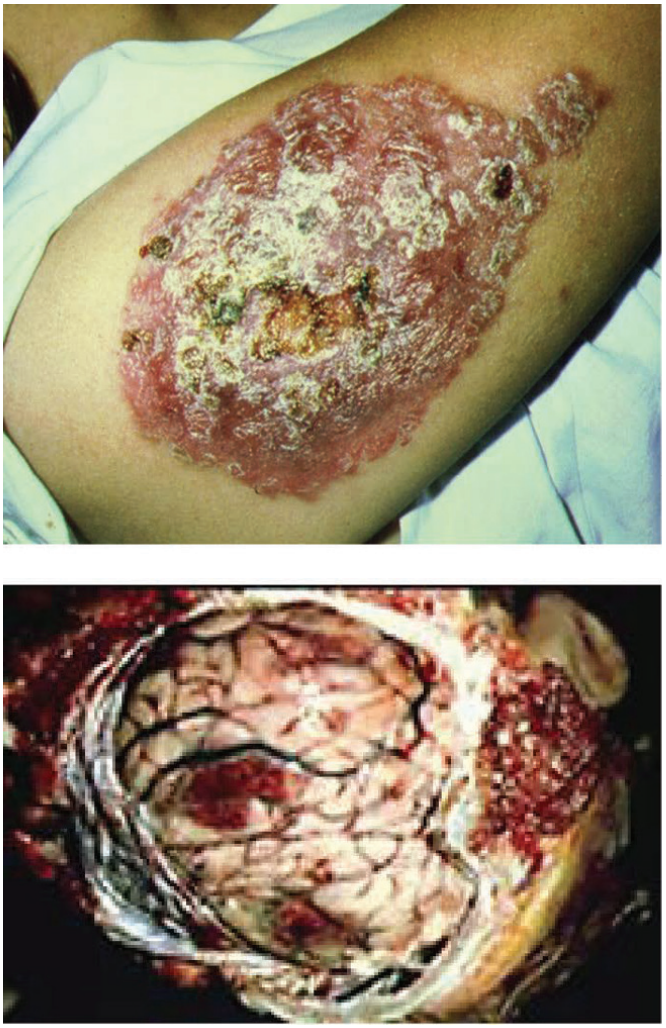


Figure 24.16 *Acanthamoeba* infection in a 28-year-old woman whose arm was bitten by her child. (Upper) Cutaneous lesion after approximately 6 months; (lower) brain lesions in same patient, disseminated disease. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photographs courtesy of G.R. Healy, CDC.)
doi:10.1128/9781555819002.ch24.f16

Other Media Approaches. Various improved medium formulations have been developed and provide some additional options. *Acanthamoeba* isolates have been recovered from clinical specimens inoculated onto buffered charcoal-yeast extract agar (BCYE), nonnutrient agar with live or dead *E. coli*, and tryptic soy agar (TSA) with 5% horse or sheep blood. Good recovery of trophozoites has been obtained on BCYE, TSA with rabbit blood, TSA with horse blood, and Remel TSA with sheep blood. BBL TSA with horse blood or rabbit blood provided good recovery of

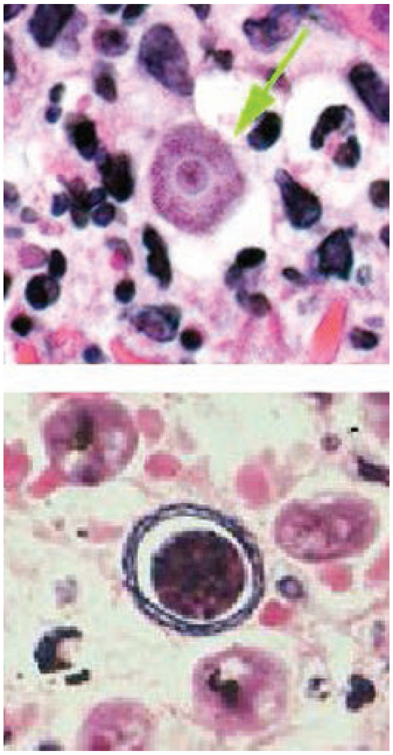


Figure 24.17 *Acanthamoeba* trophozoites and cysts in brain tissue, stained with hematoxylin and eosin. (Upper) Trophozoite (arrow). (Lower) Cyst. (Courtesy of the CDC Public Health Image Library, photographs courtesy of G. S. Visvesvara, CDC.) doi:10.1128/9781555819002.ch24.f17

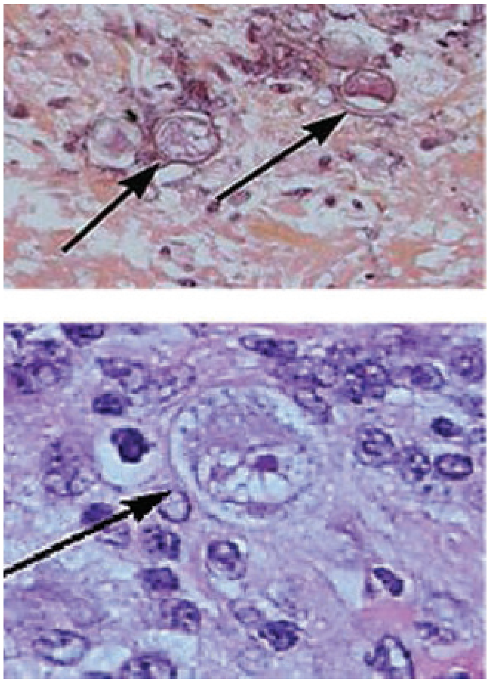


Figure 24.18 Microscopic view of *Acanthamoeba* amebae in skin. (Upper) Surgical skin biopsy specimen showing amebic cysts (arrows) in the dermal-hypodermal junction (hematoxylin and eosin stain). (Lower) Surgical skin biopsy specimen showing intravascular amebic trophozoite (arrow) characterized by acanthapodia, cytoplasmic vacuoles, and a prominent nucleolus (hematoxylin and eosin stain). (From Barete S et al, *Emerg Infect Dis* **13**[5], 2007, http://wwwnc.cdc.gov/eid/article/13/5/06-1347_article.htm.) doi:10.1128/9781555819002.ch24.f18

cysts. All species of live or dead bacteria (*Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, and *Stenotrophomonas maltophilia*) yielded good recovery of trophozoites; however, only nonnutrient agar with live *P. aeruginosa*, live *E. aerogenes*, or live *S. maltophilia* gave good recovery of cysts. TSA with rabbit blood or horse blood, BCYE, and nonnutrient agar prepared with live *P. aeruginosa*, *E. aerogenes*, or *S. maltophilia* offered optimal recovery of *Acanthamoeba*. Filtration of specimens through cellulose membranes and cultivation on nonnutrient agar plates covered with *E. coli* was compared with culture of centrifuged specimens (9). Significantly higher parasite detection could be obtained with samples processed by filtration (100% of specimens containing 10 parasites and 65% of specimens containing 2 parasites compared with 8 and 0%, respectively, with centrifugation).

Cryopreservation studies have also found that the highest viability of trophozoites of *A. culbertsoni* occurs when dimethyl sulfoxide is used at a final concentration of 10% and an equilibrium temperature of 4°C. Gross cultural or morphological changes were not noted in trophozoites thawed from frozen suspension; the organisms were grown in serum-casein-glucose-yeast extract medium (23).

Histology. Identification of acanthamoebae in ocular and other tissues can be difficult, even for trained microscopists; in histologic preparations the organisms look much like keratoplasts, as well as neutrophils and monocytes. It has been estimated that up to 70% of clinical *Acanthamoeba* keratitis cases are misdiagnosed as viral keratitis. Also, the mean time to diagnosis of keratitis due to *Acanthamoeba* infection can average 2.5 weeks longer for noncontact lens wearers than for contact lens users. Certainly the availability of rapid, accurate, and relatively simple diagnostic tests would increase the timely application of appropriate chemotherapy. Genetic markers have also been developed and used to identify pathogenic *Acanthamoeba* strains associated with nonkeratitis infections (24).

Cutaneous lesion material can be inoculated onto various growth media, including nonnutrient agar plates with a bacterial overlay. Inoculation of mammalian cell culture monolayers is also possible, as for GAE. Histologic stains such as hematoxylin and eosin, PAS, or calcofluor white

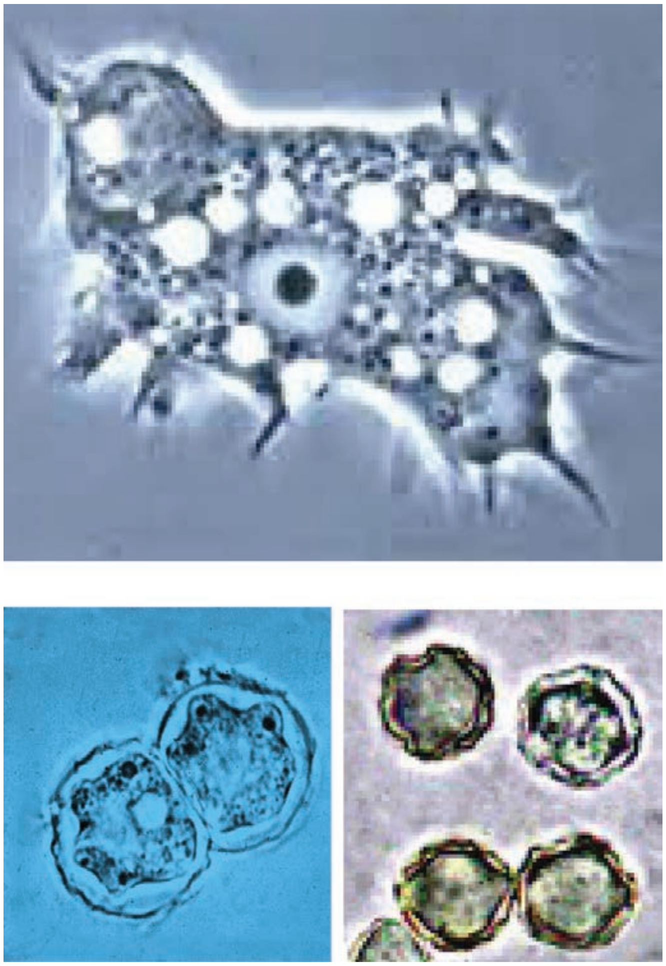


Figure 24.19 (Upper) *Acanthamoeba* trophozoite in wet preparation (note the "spiky" pseudopods, called acanthapodia). (Lower) *Acanthamoeba* cysts in wet preparation (note the double-wall appearance). (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch24.f19

can be used. Also, DNA-based molecular methods such as restriction fragment length polymorphism and randomly amplified DNA analysis can be used (25, 26).

Cytospin. CSF or bronchoalveolar lavage fluid cytospin preparations can be used to look for amebae in patients with GAE or respiratory symptoms. The characteristic morphology of the *Acanthamoeba* trophozoites, such as the prominent nucleolus, contractile vacuole, and cytoplasmic vacuoles, can be seen more easily using trichrome or hematoxylin and eosin stains on fixed preparations after cytocentrifugation.

Antibody Detection. Although detection of antibodies to *Acanthamoeba* spp. has been confirmed, the usefulness of serologic tests for the diagnosis of these infections has not been proven. Also, these procedures are not generally available. Further work related to the issue of false-positive and false-negative results and interpretive guidelines regarding past exposure or present infection must be performed before these diagnostic procedures can be routinely used.

Molecular Testing. Analysis of the 18S rRNA gene PCR product has been used to identify *Acanthamoeba* isolates from reservoirs in Taiwan (27). Molecular testing was also valuable in the investigation of an outbreak of *Acanthamoeba* keratitis, a rare, potentially blinding, corneal infection, which was detected in the United States in 2007; cases had been increasing since 2004. Although the Advanced Medical Optics Complete Moisture Plus (AMOCMP) solution was not found to be contaminated prior to opening, its anti-*Acanthamoeba* efficacy was insufficient (28).

KEY POINTS—LABORATORY DIAGNOSIS

Acanthamoeba spp.

1. As with *N. fowleri*, beware of a false-positive Gram stain (debris being misinterpreted as bacteria) (the leukocyte count in CSF may be elevated).
2. Examine clinical material on a slide with a coverslip; do not use a counting chamber (organisms in CSF within a counting chamber resemble leukocytes).
3. Nonnutrient agar with Page's saline and an *E. coli* overlay can be used for culture recovery.
4. Various other media containing different agar bases, some of which contain horse, sheep, or rabbit blood, are also available.
5. Tissue stains are also effective, and the use of calcofluor white to visualize the double-walled cyst is recommended.
6. Refer to chapter 8 for specific methods and recommendations.

Treatment

Disseminated Infections. Trophozoites and cysts of *Acanthamoeba* isolates vary in their sensitivity to antimicrobial agents. They are sensitive *in vitro* to ketoconazole, pentamidine, hydroxystilbamidine, paromomycin, 5-fluorocytosine, polymyxin, sulfadiazine, trimethoprim-sulfamethoxazole, azithromycin, and extracts of medicinal plants and, especially, to combinations of these drugs (1, 9). Increased awareness of these infections and early therapy play a large role in patient outcomes. Although steroids have been used in the past to treat cerebral edema and inflammation in CNS infections, this approach should not be used since the steroids tend to exacerbate the *Acanthamoeba* infection. Encephalitis has been treated, somewhat successfully, with antimicrobial combinations including sterol-targeting azoles (clotrimazole, miconazole, ketoconazole, fluconazole, and itraconazole), pentamidine isethionate, 5-fluorocytosine, and sulfadiazine. The use of drug combinations helps address resistance that may exist or occur during treatment. *In vitro* testing confirms strain and species differences in sensitivity, so that no single drug is effective against all amebae (29).

Amebic Keratitis. Because the infection may be misdiagnosed and/or therapy may be delayed, total blindness may result in the infected eye(s). However, if the infection is correctly diagnosed early and the epithelium alone is involved, debridement may be sufficient for cure. This approach not only removes organisms but also enhances the delivery of topical medications. However, if treatment is delayed and the organisms invade the cornea or tissues below the cornea, therapy may need to be continued for many months to a year or longer. These patients must be monitored continually because the cysts tend to be quite resistant to therapy; treatment failures tend to be fairly common. *In vitro* susceptibility testing may be very helpful in these cases (18, 29).

A topical biguanide, 0.02% chlorhexidine or polyhexamethylene biguanide (PHMB, 0.02%), either alone or combined with a diamidine, propamidine isethionate (Brolene) or hexamidine (Desomodine), has been used successfully. They are administered hourly (or alternating every half hour) day and night for the first 48 hours and then continued on a reduced schedule for days to months (30). None of these drugs is commercially available or approved for use in the United States, but they can be obtained from compounding pharmacies. Leiter's Park Avenue Pharmacy (San Jose, CA; 800-292-6773; www.leiterrx.com) is a compounding pharmacy that specializes in ophthalmic drugs. Expert Compounding Pharmacy (6744 Balboa Blvd., Lake Balboa, CA 91406; 800-247-9767) and Medical Center Pharmacy (New Haven, CT; 203-688-7064) are also compounding pharmacies. Other compounding pharmacies may be found through the National Association of Compounding Pharmacies (800-687-7850) or the Professional Compounding Centers of America (800-331-2498, www.pccarx.com). Propamidine is available over the counter in the United Kingdom and Australia. Hexamidine is available in France. Debridement is most useful during the stage of corneal epithelial infection; keratoplasty in medically unresponsive keratitis was successful in 31 eyes in 30 patients (18).

Cutaneous Disease. Cutaneous infections with subsequent dissemination to other body sites have been found in patients who are immunocompromised due to HIV infection or preparation for organ transplantation. As with other disease manifestations, a number of drug combinations have been tried, some of which were successful. However, not all combinations are effective; toxicity with certain drugs also becomes a consideration (Table 24.7).

In a case of skin ulcers due to *Acanthamoeba* in an immunodeficient individual who had undergone kidney transplantation, the patient was successfully treated after extended therapy over a period of 8 months. He received topical as well as systemic administration of combination therapy. The skin ulcers were cleaned twice daily with chlorhexidine gluconate solution, and 2% ketoconazole cream was applied topically. He also received pentamidine isethionate intravenously for 1 month and was then given oral itraconazole therapy for 8 months. This resulted in complete healing of the ulcers (6).

Successful treatment of *Acanthamoeba* infections has been limited; specific examples of patients, disease manifestations, treatment, and outcomes are seen in Table 24.7.

Epidemiology and Prevention

General Preventive Measures. General preventive measures are similar to those for *N. fowleri*. Several disinfectants have been tested against both *Naegleria* and *Acanthamoeba* spp. The conclusion is that *Naegleria* spp. are generally susceptible to swimming-pool levels of chlorine but *Acanthamoeba* spp. are more resistant (6). The recovery of *Acanthamoeba* spp. in nasal isolates and pharyngeal swabs may indicate human introduction of the organisms into swimming pools. With the increasing reports of infection in wearers of soft contact lenses, it will be important to carefully consider the effectiveness of various contact lens disinfection systems. A number of factors probably play a role in the increased incidence of infection: a large number of HIV-infected individuals and more patients undergoing cancer chemotherapy or immunosuppressive therapy for organ transplantation.

Mitochondrial DNA fingerprinting of *Acanthamoeba* spp. from the American Type Culture Collection and environmental sources has confirmed that approximately half of the 35 isolates displayed fingerprints similar to those of clinical isolates. Comparisons with other published mitochondrial DNA fingerprints indicated that two groups have counterparts in Europe and Japan and in Europe and Australia. These data provide strong evidence that the most common clinical isolates do have counterparts that are geographically widespread and can be isolated from the environment.

Acanthamoeba cysts can remain viable in water at 4°C for 24 years and have survived desiccation for 20 years (31). In animal studies, it has been shown that some of the isolates completely lost their virulence only after 8 years of *in vitro* cultivation. On the basis of these results, one can assume that cyst viability may be as long as 25 years and that these cysts can maintain their invasive properties (6, 8).

Contact Lens Care. In one recent study of marketed contact lens disinfection solutions against keratitis-causing *Acanthamoeba castellanii*, none of the contact lens disinfection solutions exhibited cysticidal effects using qualitative assays; i.e., cysts treated with these solutions reemerged as viable amebae upon inoculation in the growth medium.

TABLE 24.7 *Acanthamoeba* infections, treatment, and positive patient outcomes[a]

Immunocompetent or underlying disease	Amebic disease involvement	Treatment[b]	Outcome
Immunocompetent	CNS (2 patients)	TMP/SMZ + KC + rifampin	Survived
AIDS	Cutaneous/sinusitis	Ketoconazole + i.v. FC	Resolution of lesions; no CNS involvement
AIDS	Cutaneous/sinusitis	Debridement of sinuses + pentam + topical chlorhexidine + 2% KC; pentam toxicity, maintenance on FC	Survived
AIDS	CNS lesion	Sulfadiazine + pyrimethamine, flucon + sulfadiazine; one localized brain lesion excised	Survived
AIDS	Rhinosinusitis	Removal of all diseased nasal mucosa; pentam, levofloxacin, FC, AmpB	Survived
HIV positive	Sinusitis	Debridement of sinuses, IT + gentamicin nasal wash	Resolution of lesions
HIV positive	Sinusitis/cutaneous, lobular panniculitis	Surgical debridement + IT, azithromycin, 5FC, rifampin	Survived
Renal transplant	Cutaneous lesions, IgA deficiency	Pentamidine + chlorhexidine + 2% KC cream; maintenance on oral IT	Resolution of lesions; no CNS involvement
Lung transplant	Cutaneous	AmpB + i.v. pentam + IT + chlorhex/KC cream + azithro + FC + pentam, maintenance FX + clarithromycin	Survived, but died 6 months after *Acanthamoeba* diagnosis

[a] Based on references 6–9, 18, 29, 30.

[b] FC, fluorocytosine; IT, itraconazole; azithro, azithromycin; AmpB, amphotericin B; KC, ketoconazole; TMP/SMZ, trimethoprim-sulfamethoxazole; pentam, pentamidine; flucon, fluconazole; chlorhex, chlorhexidine; i.v., intravenous.

These data indicate possible serious sequelae for contact lens users (32).

Both failure to disinfect daily-wear soft contact lenses and the use of chlorine-release lens disinfection systems, which have little effect against the organism, are major risk factors for *Acanthamoeba* keratitis. These risks have been very common in disposable-lens use. Some investigators think that over 80% of cases of *Acanthamoeba* keratitis could be avoided by the use of lens disinfection systems that are effective against the organism.

Current recommendations for contact lens wearers include (i) regular scrubbing of contact lens case interiors to disrupt biofilms, (ii) exposure of the contact lens case to very hot water (at least 70°C) to kill *Acanthamoeba* contaminants, (iii) allowing the contact lens case to air dry between uses, (iv) use of a two-step system if hydrogen peroxide disinfection is preferred, and (v) regular replacement of the contact lens case. It has also been documented that some bottled waters contain large numbers of potential ocular pathogens and that, as a general recommendation, bottled water is not safe for routine use with contact lenses.

Pathogenic Bacteria Carriage within Free-Living Amebae. There have been a number of studies reviewing the potential for carriage of pathogenic bacteria within free-living amebae. In one study in Germany, *Legionella*-contaminated hot water systems and moist sanitary areas in six hospitals were sampled for amebae. Amebae were detected in 29 of 56 hot-water samples and 23 of 49 swabs; six *Acanthamoeba* species isolated from the moist areas were considered potential pathogens (11). It has also been shown that *Acanthamoeba* species can produce vesicles containing live *Legionella pneumophila* cells. Such vesicles may be agents for the transmission of legionellosis associated with cooling towers, and the risk may be underestimated by plate count methods since each vesicle could contain several hundred bacteria. Data also demonstrate that obligate anaerobic bacteria can survive and multiply in *Acanthamoeba* spp. under aerobic conditions, thus suggesting a previously undescribed mechanism for the spread, replication, and persistence of obligate anaerobes in the environment (11). *E. coli* O157 also survives and replicates within *A. polyphaga*. These amebae may serve as an important environmental reservoir for transmission of *E. coli* O157 and other pathogens (11).

The incidence of lung and other diseases due to nontuberculous mycobacteria (NTM) is increasing. NTM sources include potable water, especially in households where NTM populate pipes, taps, and showerheads. NTM share habitats with free-living amebae and can grow in these amebae as parasites or as endosymbionts. Free-living amebae containing NTM may form cysts that protect mycobacteria from disinfectants and antibiotics (33).

Balamuthia mandrillaris

The free-living ameba *Balamuthia mandrillaris* is relatively uncommon and was originally thought to be another harmless soil organism, unlikely or unable to infect mammals. However, since *B. mandrillaris* was first discovered in 1986 in a pregnant mandrill at the San Diego Wild Animal Park that died of meningoencephalitis, a number of primates including gorillas, gibbons, baboons, orangutans, and monkeys, as well as a sheep and a horse, have died of CNS infection caused by this organism. Over 200 cases of human amebic encephalitis worldwide have been identified, with >30 being seen within the United States (3, 34). Death can occur from 1 week to several months after the onset of stroke-like symptoms, which can mimic other conditions including brain stem glioma. Patients eventually die of a massive CNS infection. One individual who survived the infection was left with severe neurologic deficits.

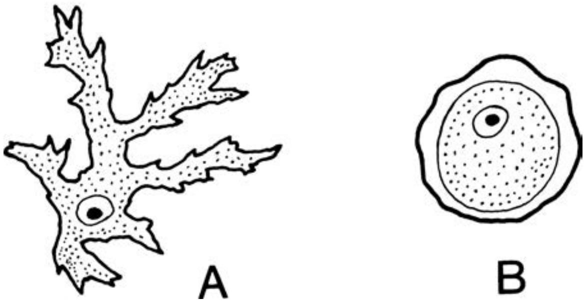
Balamuthia spp. produce virulence factors that enhance binding to and penetrating the blood brain barrier. These organisms can cause cell death by direct phagocytosis as well as through various toxins and proteases capable of destroying the extracellular matrices. Human-to-human transmission has been documented through solid organ transplantation in 2009 and 2010 (34).

Life Cycle and Morphology

The life cycle of *B. mandrillaris* has two stages, cysts and trophozoites. The trophozoites can gain entry to the body through the respiratory tract or through a skin defect. Trophozoites are capable of hematogenously disseminating to the CNS. This pathogen is capable of producing GAE which is a very rare cause of fulminant life-threatening meningoencephalitis.The life cycle is similar to that seen with *Acanthamoeba* spp.; like *Acanthamoeba* spp., *Balamuthia* does not have a flagellated stage in its life cycle as do organisms classified as *N. fowleri* (Fig. 24.20). Unlike *Acanthamoeba*, which is widely distributed in the environment and can be isolated from soil, water, and even air, *B. mandrillaris* is more difficult to isolate and to culture, with a generation time ranging from 20 to 50 h (35).

Morphology of Trophozoites. Trophozoites of *B. mandrillaris* are usually irregular in shape, and actively feeding amebae may measure from 12 to 60 µm in length (normal, 30 µm) (Fig. 24.21). As a eukaryotic cell, *B. mandrillaris* possesses a nucleus but may possess more than one nucleolus. In tissue culture, broad pseudopodia are usually seen; however, as the monolayer cells are destroyed, the trophozoites develop fingerlike pseudopodia.

Figure 24.20 (A) Trophozoite and (B) cyst of *Balamuthia mandrillaris*. (Illustration by Sharon Belkin.)
doi:10.1128/9781555819002.ch24.f20



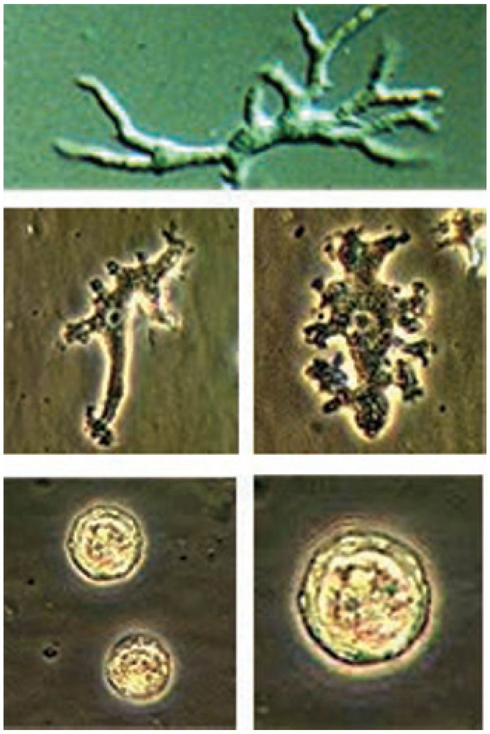


Figure 24.21 *Balamuthia mandrillaris*. (Top and middle) Trophozoites; note the finger-like pseudopodia. (Bottom) Cysts. (Courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch24.f21

Morphology of Cysts. Cysts are somewhat smaller than the trophozoites, are usually spherical, and measure from 13 to 30 µm in diameter (Fig. 24.20). Under electron microscopy, the cysts are characterized by having three layers in the cyst wall: an outer wrinkled ectocyst, a middle structureless mesocyst, and an inner thin endocyst. Under light microscopy, they appear to have two walls, an outer irregular wall and an inner round wall.

Clinical Disease

The disease is very similar to GAE caused by *Acanthamoeba* spp. and has an unknown incubation period. The clinical course tends to be subacute or chronic and is usually not associated with swimming in freshwater. No characteristic clinical symptoms, laboratory findings, or radiologic indicators have been found to be diagnostic for GAE. The neuroimaging findings show heterogeneous, hyperdense, nonenhancing, space-occupying lesions.

Whether single or multiple, they involve mainly the cerebral cortex and subcortical white matter. These findings suggest a CNS neoplasm, tuberculoma, or septic infarcts. Patients complain of headaches, nausea, vomiting, fever, visual disturbances, dysphagias, seizures, and hemiparesis. There may also be a wide range in terms of the clinical course, from a few days to several months. If the route of entry is through broken skin, painless nodules may be observed, developing into lesions containing trophozoites while the patient is alive.

Unlike *Acanthamoeba* encephalitis, *Balamuthia* amebic encephalitis has been found in immunocompetent individuals (individuals who were negative for syphilis, diabetes mellitus, malignancies, and fungal, HIV type 1, HIV type 2, and mycobacterial infections and had normal CD4- and CD8-positive T-lymphocyte counts and B-lymphocyte counts). These findings confirm the virulent nature of this pathogen. In immunocompetent hosts, an inflammatory response is mounted and amebae are surrounded by macrophages, lymphocytes, and neutrophils. However, with rare exceptions, these patients also tend to die of severe CNS disease.

Both trophozoites and cysts of *B. mandrillaris* are found in many of the same CNS tissues as are *Acanthamoeba* spp. Although differentiation of these two organisms in tissue by light microscopy is difficult, *B. mandrillaris* may have more than one nucleolus in the nucleus in some tissue sections (1) (Fig. 24.22). Generally, electron microscopy and histochemical methods are required for definitive identification of *B. mandrillaris*. PCR or an immunofluorescence test using species-specific sera is the most reliable means of distinguishing between *Acanthamoeba* and *Balamuthia* spp.

Magnetic resonance imaging and computerized tomography are useful and will exhibit brain lesions, which are found in the parietal lobe, anterior lobe, temporal lobe, and cerebellum. Lesions have been described as calcified and as forming a mass-like structure. Biopsy may reveal a necrotic cortex and the faint outline of perivascular monocytes. Giemsa stain shows both trophozoites and cysts and may be used to stain wet mounts, while calcofluor white preferentially stains cysts (has a binding affinity for cellulose, chitin, and potentially other glycans), which may aid in identification. However, without an early diagnosis and high index of suspicion, prognosis remains poor, with a case-fatality rate of >98%.

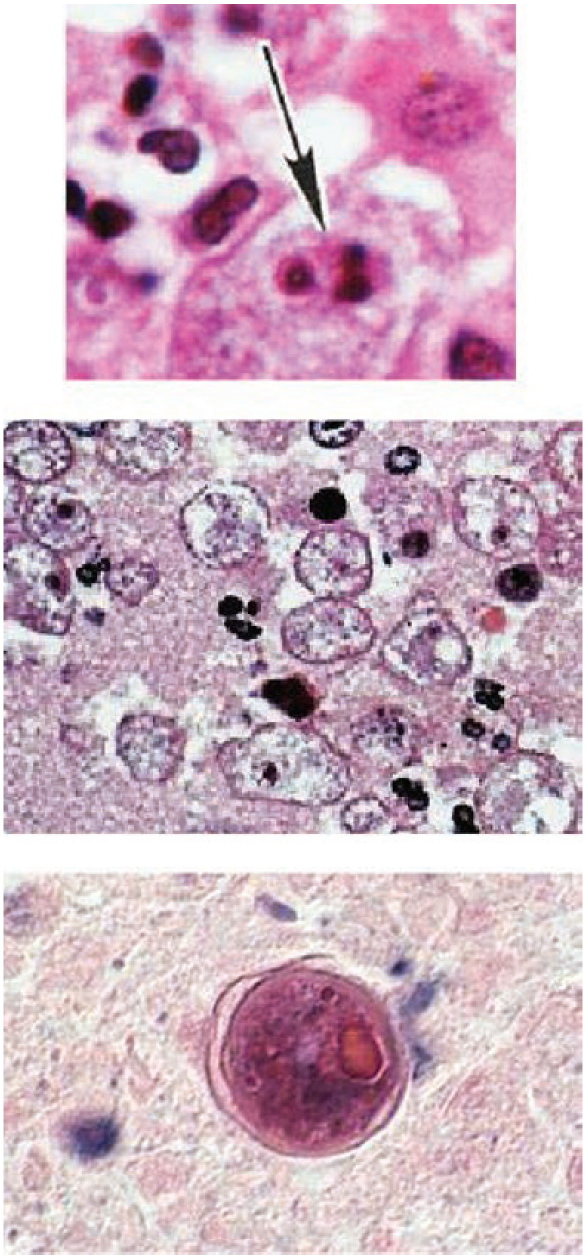


Figure 24.22 *Balamuthia mandrillaris*. (Top and middle) Trophozoites in brain. (Bottom) Cyst in brain (hematoxylin and eosin). (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch24.f22

Diagnosis

It is interesting that attempts to isolate the organisms from some humans with GAE have not been successful, and *B. mandrillaris* has also been shown not to grow well on *E. coli*-seeded nonnutrient agar plates. In the diagnostic laboratory, these organisms can be cultured in mammalian cell cultures; some success has been obtained with monkey kidney cells and with MRC, HEp-2, and diploid macrophage cell lines. Using human brain microvascular endothelial cells, investigators have cultured *B. mandrillaris* postmortem from brain and CSF from a case of granulomatous amebic meningoencephalitis (36). A cell-free growth medium is also now available (1). The trophozoites are characterized by extensive branching and a single nucleus (occasionally binucleate forms are seen) with a central karyosome. Occasionally, a few elongated forms with several contractile vacuoles are seen. The cysts have a single nucleus (occasionally, binucleate forms are seen) and have the typical double wall, with the outer wall being thick and irregular. The identity of *B. mandrillaris* can be confirmed using immunofluorescence staining assays and PCR methods.

Serum antibodies to *B. mandrillaris* have been found in both adults and children; however, testing is not currently available for routine use. Although the mouse model is rarely used and is somewhat impractical, mice infected with *Acanthamoeba* spp. or *B. mandrillaris* may die of acute disease within 5 to 7 days or chronic disease after several weeks. In either case, amebae can be seen in the mouse brain after culture or histologic examination.

Based on sequence analysis of mitochondrial small-subunit rRNA genes, primers have been developed that amplify a *Balamuthia*-specific PCR product. These primers will be useful not only for retrospective analyses of preserved tissues but also for possible organism identification *in vivo* (2). Genotyping studies indicate that lethal infections caused by *B. mandrillaris* are due to a single species with a global distribution. Apparently, there is no correlation between a particular mitochondrial sequence and the genus of the infected vertebrate. The mitochondrial sequence from the mandrill isolate is identical to that obtained from a human (6).

Treatment

In vitro studies indicate that *B. mandrillaris* is susceptible to pentamidine isethiocyanate and that patients with this infection may benefit from this treatment in combination with other drugs. However, pentamidine is associated with adverse effects including nephrotoxicity, leukopenia, elevated liver enzymes, and hypoglycemia. Also, pentamidine does not cross the normal, intact blood-brain barrier well (18). Other studies indicate that sulfadiazine, azithromycin, clarithromycin, flucytosine, and miltefosine have amebicidal activity (18). In one immunocompetent patient who survived, treatment included combination antibiotics (pentamidine, 300 mg intravenously once a day; sulfadiazine, 1.5 g four times a day; fluconazole, 400 mg once a day; and clarithromycin, 500 mg three times a day), with no neurologic sequelae seen (21). See chapter 36 for additional information.

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Routes of CNS entry may include ameba penetration of the olfactory neuroepithelium via the nasal route. However, hematogenous dissemination from a primary lung (ameba entry via the respiratory tract) or skin (ameba entry through breaks in the skin followed by exposure to contaminated soil, e.g., working in gardens) entry is thought to be more common. Current recommendations are similar to those for *Acanthamoeba* spp. Although attempts to isolate *B. mandrillaris* from soil samples related to previous encephalitis cases were unsuccessful, there was always some question regarding the designation “free-living” amebae. However, following the death of a 3-year-old Northern California resident from amebic encephalitis caused by *Balamuthia*, environmental samples were collected around the child’s home and outdoor play areas. An ameba, consistent with *Balamuthia*, was isolated from the soil found in a potted plant in the home (3). Thus, the isolation of *Balamuthia* amebae from soil affirms its status as a free-living ameba; like *Acanthamoeba* and *Naegleria*, *Balamuthia* is also an opportunistic pathogen.

From the information presented above, it is clear that *B. mandrillaris*, formerly regarded as having no pathogenic potential, will continue to be identified as the etiologic agent of fatal meningoencephalitis in humans as well as animals (Fig. 24.20 to 24.22). This opportunistic infection may also continue to cause disease in individuals who are immunocompromised, as well as those who are immunocompetent.

Sappinia (*diploidea*) *pedata*

The genus *Sappinia* is a newly recognized human pathogen causing amebic encephalitis; there are two species, *S. diploidea* and *S. pedata*. An infection with *S. pedata* has been well documented in a single patient, who survived the infection, while it is not known if *S. diploidea* can cause human infections (37, 38). Trophozoites measure from 40 to 70 µm and have a distinctive double nucleus (Fig. 24.23). Transmission electron microscopy confirms that the trophozoites contain two nuclei attached to each other by connecting perpendicular filaments. The trophozoites ingested host blood cells and stained brightly with Giemsa and PAS stains. Cysts were not seen, but their presence in the human host was not excluded, and they have been seen in culture. These and other morphologic characteristics led to the diagnosis of *S. diploidea*, a demonstrated human pathogen, for the first time. However, based on molecular techniques (real-time PCR assays based on 18S rRNA gene sequences), the organism was reidentified as *S. pedata*. The assay specific for *Sappinia* can be incorporated into the multiplex PCR assay described by

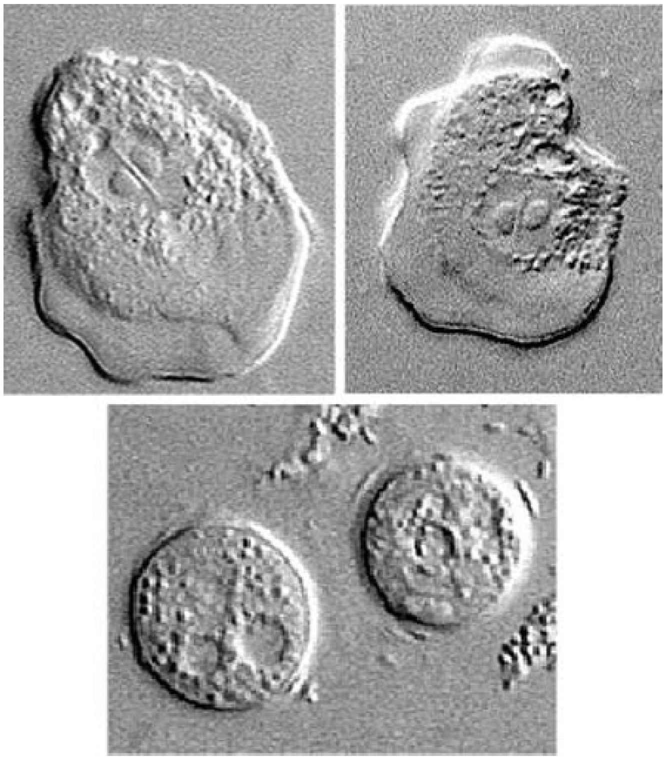

Figure 24.23 *Sappinia* sp. using differential interference contrast (DIC) microscopy. (Upper) Trophozoites; note the presence of two nuclei, connected to each other by connecting filaments. (Lower) Cysts. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch24.f23

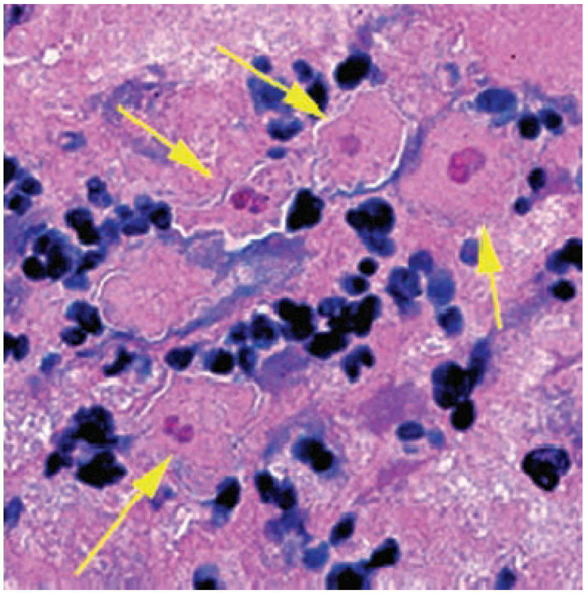

Figure 24.24 *Sappinia pedata*. Four trophozoites (arrows) seen in brain tissue. Note the presence of two nuclei within some of the trophozoites where both are in the same plane of focus. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch24.f24

TABLE 24.8 Summary of information on the newly recognized pathogenic free-living ameba *Sappinia pedata*[a]

Characteristic	Comments
Patient	38-year-old man
Symptoms	Visual disturbances, headache, seizure
Brain scan	Solitary tumor-like mass in posterior left temporal lobe
Surgical excision	Necrotizing hemorrhagic inflammation
Histologic findings	Central necrotic and hemorrhagic inflammations; acute and chronic inflammatory cells without granulomas or eosinophils; trophozoites present in viable brain parenchyma on mass periphery without inflammatory response
Organisms present	Amebic trophozoites
Trophozoite characteristics	Large (40–70 μm), 2 nuclei per trophozoite
Stained trophozoites	Bright; Giemsa, periodic acid-Schiff stains
Cysts	Obtained in culture; not found in tissue
Therapy after surgery	Azithromycin, pentamidine, itraconazole, flucytosine

[a] Information from reference 37.

Qvarnstrom et al., which distinguishes *Acanthamoeba*, *B. mandrillaris*, and *N. fowleri* for simultaneous detection of the four genera of free-living amebae that cause infections in humans (39).

The patient was a 38-year-old man who complained of visual disturbances and headache; he also had a seizure. He had reported a sinus infection prior to the onset of symptoms. Brain scans revealed a solitary tumor-like mass in the posterior left temporal lobe. On biopsy, the mass demonstrated necrotizing hemorrhagic inflammation, which contained free-living amebae (Fig. 24.24). After surgical excision of the tumor-like mass, the patient was treated with a sequential regimen of antiamebic drugs, including azithromycin, pentamidine, itraconazole, and flucytosine. Characteristics of this case are summarized in Table 24.8.

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25

Protozoa from Other Body Sites

Trichomonas vaginalis
Toxoplasma gondii

Trichomonas vaginalis

Trichomonas vaginalis was first isolated in 1836 from the purulent discharge from female genital organs. The infection occurs worldwide and has been found in the population whenever the appropriate specimens have been examined for the presence of the organism. It is estimated that 5 million women and 1 million men in the United States have trichomoniasis, with the annual incidence of new cases estimated at 7.4 million (1). The annual incidence of trichomoniasis worldwide is estimated to be more than 275 million cases, which does not include the number of asymptomatic cases that are not treated. In North America, more than 8 million new cases are reported yearly, with an estimated rate of asymptomatic cases being as high as 50% (1). Trichomoniasis is now the primary nonviral sexually transmitted infection (STI) worldwide. In women, disease manifestations may include vaginitis, cervicitis, urethritis, pelvic inflammatory disease, and adverse birth outcomes. *T. vaginalis* infection may also increase risk of HIV transmission, the incidence and severity of cervical and prostate cancer, and increased infertility. Some of the most recent developments in our understanding of *T. vaginalis* pathobiology include the increased association with HIV in terms of exposure to STIs and a positive association with aggressive prostate cancers (2).

Population genetic analysis has shown that *T. vaginalis* is a genetically diverse parasite that consists of two types present in equal proportions worldwide. Parasites belonging to the two types (type 1 and type 2) differ significantly in the rate at which they harbor the *T. vaginalis* virus, a double-stranded RNA virus implicated in parasite pathogenesis, and in their sensitivity to the widely used drug metronidazole (3). The extensive clinical variability and disease sequelae are matched by significant genetic diversity in the organism itself; this strongly suggests a connection between the genetic identity of isolates and their clinical manifestations (4).

T. vaginalis has the largest protozoan genome known, potentially encoding approximately 60,000 proteins. To what degree these genes are expressed is not well known, and only a few transcription factors and promoter domains have been identified.

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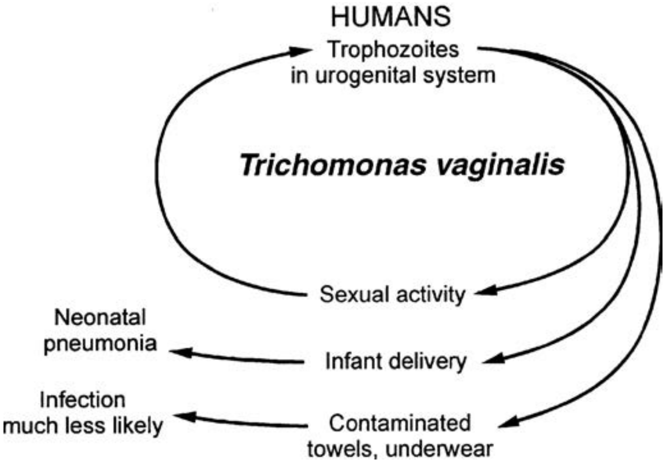

HUMANS
Trophozoites in urogenital system
Trichomonas vaginalis
Sexual activity
Neonatal pneumonia
Infant delivery
Infection much less likely
Contaminated towels, underwear

Figure 25.1 Life cycle of *Trichomonas vaginalis*. doi:10.1128/9781555819002.ch25.f1

Life Cycle and Morphology

T. vaginalis exists as flagellate and ameboid trophozoites and the pseudocystic stages (5, 6) (Fig. 25.1). Pseudocyst forms of *T. vaginalis* isolated from cervical neoplasia patients demonstrated distinct, different, and significant *in vitro* growth profiles when grown in cultures from day 1 up to day 5 as compared with the same life cycle stages isolated from noncervical neoplasia but symptomatic patients. The exposure of oxygen-adapted parasites to human epithelial cells primarily induces cytoskeletal rearrangement and proliferation, with a rapid transition from spindle-shaped flagellates to tissue-feeding and actively dividing ameboids (6). As an extracellular parasite residing in the urogenital tract, *T. vaginalis* must adhere to epithelial cells as an initial step towards colonizing the host and establishing infection. The flagellate trophozoite is 7 to 23 µm long and 5 to 15 µm wide (Fig. 25.1; Table 25.1). The axostyle is usually extremely obvious, and the undulating membrane stops halfway down the side of the trophozoite (Fig. 25.2 and 25.3). The nuclear chromatin is uniformly distributed, and there are many siderophil granules that are particularly evident along the axostyle. The axostyle is thought to anchor the parasite to vaginal epithelial cells. The cytoskeleton is composed of tubulin and actin fibers.

There are five flagella, with the fifth being incorporated within the undulating membrane. Different conditions alter the shape of the trophozoites; in axenic culture the trophozoite tends to be more pear shaped and oval, but it may appear more ameboid when attached to vaginal epithelial cells. When environmental conditions are unfavorable, the organisms may tend to round up and internalize the flagella.

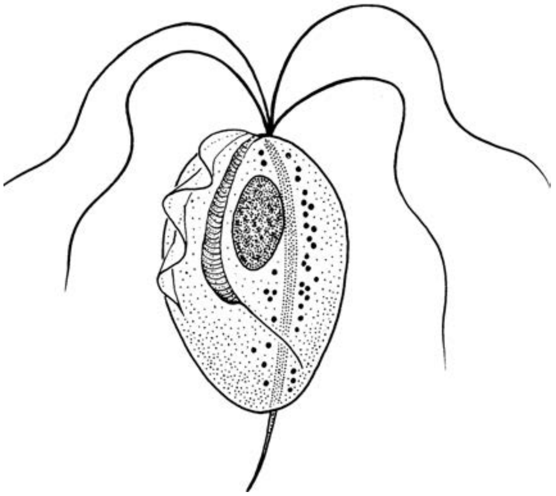
The life cycle has been described but is not totally defined. In growth-phase culture, large, round forms of the organism are seen, some without flagella, some with flagella and a dividing nucleus, and some with flagella and multiple nuclei. These forms are probably developmental stages that appear prior to the typical mononuclear flagellates that generally reproduce by longitudinal binary fission. The urogenital trichomonads vary in size and shape. Physicochemical conditions (e.g., pH, temperature, oxygen tension, and ionic strength) affect the shape of trichomo-

TABLE 25.1 Characteristics of *Trichomonas vaginalis* (primarily trophozoites)[a]

Shape and size: trophozoite	Pear shaped; 7–23 µm long (avg, 13 µm), 5–15 µm wide
Shape and size: pseudocyst	Round, no motility, apparently no true cyst wall
Shape and size: ameboid form	Various, pseudopod extension, flagella still visible
Motility	Jerky, rapid
No. of nuclei and visibility	1; not visible in unstained mounts
No. of flagella (usually difficult to see)	3–5 anterior, 1 posterior
Other features	Seen in urine, urethral discharges, and vaginal smears; undulating membrane extends half the length of the body; no free posterior flagellum; axostyle easily seen
Infective stage	Trophozoite
Usual location	Vagina (male, urethra)
Striking clinical findings	Leukorrhea, pruritus vulva (thin, white urethral discharge in male)
Other sites of infection	Urethra (prostate in male)
Stage usually recovered during clinical phase	Trophozoite only (no cyst)

[a] Adapted from references 1, 5, and 7.

Figure 25.2 *Trichomonas vaginalis* trophozoite. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch25.f2



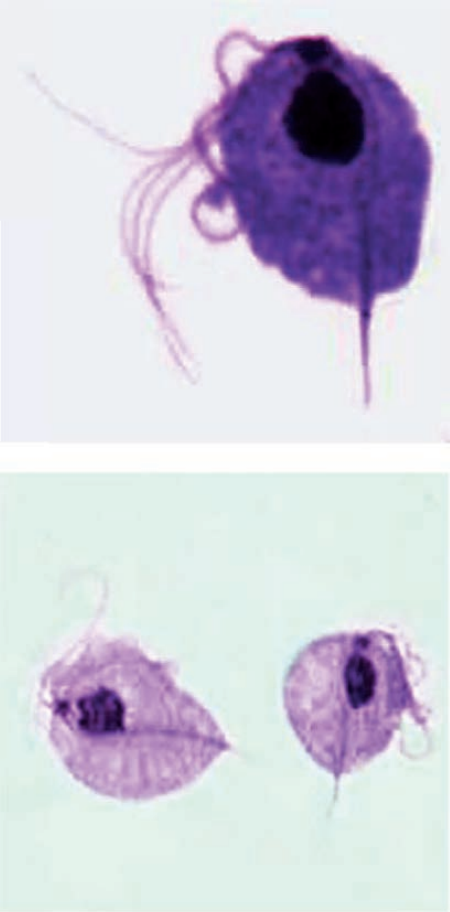


Figure 25.3 *Trichomonas vaginalis* trophozoites; Giemsa stain. (Lower, courtesy of CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch25.f3

nads; however, shape tends to be more uniform among cells grown in nonliving culture media than among those observed in vaginal secretions and urine (7) (Fig. 25.4).

Glucose Metabolism. *T. vaginalis* is a primitive eukaryotic organism; however, its metabolism is similar to that of primitive anaerobic bacteria (8). Carbohydrate metabolism is described as being fermentative under both anaerobic and aerobic conditions because glucose is incompletely oxidized. Carbohydrate metabolism occurs in the cytoplasm and an organelle called the hydrogenosome, which is similar to the mitochondria of higher eukaryotes. Within the cytoplasm, glucose is converted to phosphoenolpyruvate and subsequently to pyruvate via the typical Embden-Meyerhof-Parnas pathway. Pyruvate, which is generated through glycolysis, undergoes further metabolism in the hydrogenosome. The pyruvate:ferredoxin oxidoreductase has retained diversity in the usage of alternative keto acids for energy production by using a wide variety of substrates. Also, *T. vaginalis* has alternative enzymes that are active in metronidazole-resistant parasites; these alternative pathways are clearly important for the survival of the organism (8). Although *T. vaginalis* is not very energy efficient, it can adapt its metabolism to variable carbon sources. The organism has a high maintenance energy, expending up to half its carbon flow on maintaining internal homeostasis. This ability is critical since the vaginal environment is constantly changing in terms of pH, hormones, menses, and the nutrient supply (8).

Surface carbohydrates have also been implicated in the adhesion of *T. vaginalis* to epithelial cells. The most abundant surface glycoconjugate from the parasite has been described as a lipophosphoglycan, which has also revealed a role in immune interaction. However, extensive studies have demonstrated that *T. vaginalis* lipophosphoglycan-like material does not contain significant amounts of phosphate in the glycan core and should probably be renamed *T. vaginalis* lipoglycan (9).

Lipid Metabolism. *T. vaginalis* contains cholesterol, phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin as its major phospholipids; apparently the organism is unable to synthesize fatty acids and sterols and must rely on exogenous sources of lipid moieties to survive. It has been postulated that *de novo* glycolipid and glycophosphosphingolipid synthesis occurs in trichomonads.

Amino Acid Metabolism. Although carbohydrates are the preferred source of nutrients, amino acids can sustain survival and growth. Under normal culture conditions, *T. vaginalis* consumes large amounts of arginine and smaller amounts of methionine for energy production. There appears to be some form of equilibrium that is maintained between the free amino acids within the cell and its external environment.

Nucleotide Metabolism. The organism is unable to synthesize purines and pyrimidines; adenine and guanine or their nucleosides are required for growth, as are thymidine, cytidine, uracil, and/or uridine (8). It also appears that there may be two nucleoside carriers, a characteristic that has also been found in *Leishmania donovani*.

Humans are the only natural host for *T. vaginalis*, and normal body sites for these organisms include the vagina and prostate. Apparently, the organisms feed on the mucosal surface of the vagina, where bacteria and leukocytes are found (1). The preferred pH for good growth in females is slightly alkaline or acidic (pH 6.0 to 6.3 optimal), not the normal pH of the healthy vagina. Although the organisms can be recovered in urine, in urethral discharge, or after prostatic massage, the pH preference of the organisms in the male has not been determined. Often, the organisms are recovered in the centrifuged urine sediment from both male and female patients.

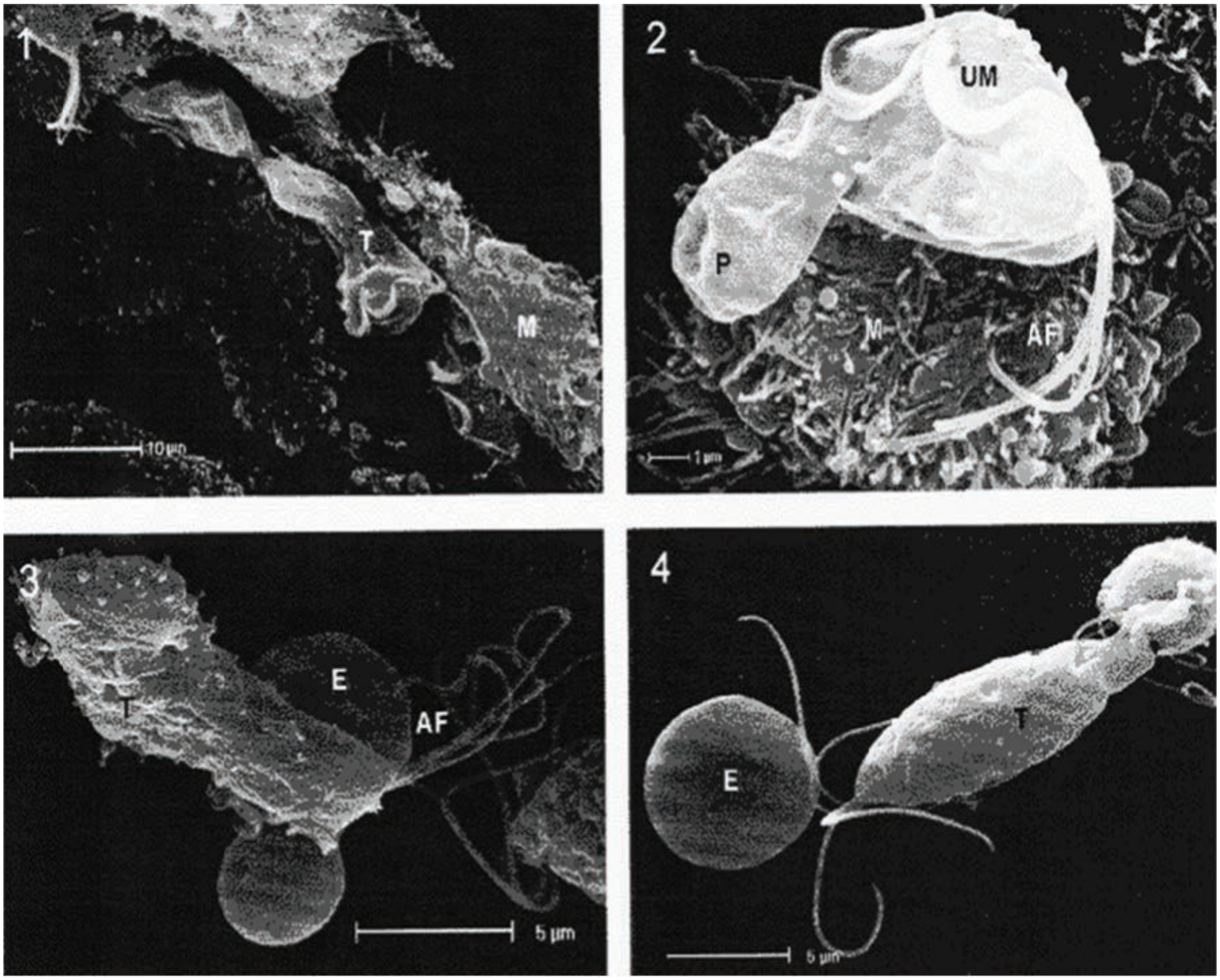


Figure 25.4 *Trichomonas vaginalis* trophozoites and ameboid forms. (1) Several ameboid trophozoites of *Trichomonas vaginalis* are attached to one another by their pseudopods, forming a group of organisms. M, McCoy cell; T, trichomonad. (2) Pseudopodia-like extensions exhibited by *Trichomonas vaginalis*. Note the parasite attached to the McCoy cell by its ventral surface. AF, anterior flagella; M, McCoy cell; P, pseudopodia-like extensions; UM, undulating membrane. (3) Trophozoite of *Trichomonas vaginalis* applied to an erythrocyte after 30 min of interaction between parasites and erythrocytes. E, erythrocyte; T, trichomonad. (4) Ameboid trophozoite of *Trichomonas vaginalis* after 60 min of interaction between parasites and erythrocytes. E, erythrocyte; T, trichomonad. (Courtesy of reference 7.)
doi:10.1128/9781555819002.ch25.f4

Clinical Disease

T. vaginalis is site specific and usually cannot survive outside the urogenital system. Although the incubation period is not well defined, *in vitro* studies provide a range of 4 to 28 days. After introduction, proliferation begins, with resulting inflammation and large numbers of trophozoites in the tissues and the secretions. As the infection becomes more chronic, the purulent discharge diminishes, with a decrease in the number of organisms. The onset of symptoms such as vaginal or vulval pruritus and discharge is often sudden and occurs during or after menstruation as a result of the increased vaginal acidity. In acute infections, diffuse vulvitis is seen and is due to copious leukorrhea. The discharge is frothy, yellow or green, and mucopurulent; however, only about 10 to 12% of women exhibit this frothy discharge. Small punctate hemorrhagic spots can be seen on the vaginal and cervical mucosa; this has been called a “strawberry appearance” and is seen in about 2% of patients. In general, symptoms include vaginal discharge (42%), odor (50%), and edema or erythema (22 to 37%) (1). Other complaints include dysuria and lower abdominal pain. Vaginal trichomoniasis may be associated with bacterial vaginosis, candidiasis, gonorrhea, and syphilis. Concurrent infections with herpes simplex virus and human papillomavirus are also seen.

In chronic infections, symptoms may be very mild with pruritus and some pain during sexual intercourse, while the vaginal secretion may be scanty and mixed with mucus. Individuals with these symptoms are the major source of transmission. From 25 to 50% of infected women may be asymptomatic and have a normal vaginal pH of 3.8 to 4.2 and normal vaginal flora. Although there is a carrier form, about 50% of these women will develop clinical symptoms during the following 6 months.

Although vaginitis is the most common finding in women with trichomoniasis, other complications include pyosalpinx (distention of a fallopian tube with pus), endometritis, infertility, low birth weight, and cervical erosion. There is also an association with increased HIV transmission and cervical dysplasia (1, 10). In a study of Malawian men, there was no difference in the rate of HIV seropositivity in men with and without *T. vaginalis* infection. However, for HIV-positive men with symptomatic urethritis, the median HIV RNA concentration in seminal plasma was significantly higher in patients with trichomoniasis than in those without trichomoniasis (1).

About 20% of women with vaginal trichomoniasis have dysuria, a symptom that may occur before any additional symptoms appear. Infection in the male may be latent, with essentially no symptoms, or may be present as self-limited, persistent, or recurring urethritis. *T. vaginalis* has been detected in 10 to 20% of subjects with nonspecific urethritis and in 20 to 30% of those whose sexual partners had vaginitis. Once established, the infection persists for a long time in females but only for a short time (usually about 10 days or less) in males.

The prevalence of *T. vaginalis* in males has not been studied as extensively as in females. Estimates vary from 4 to 20% of men with urethritis to 20 to 40% of male sexual partners of infected females. *T. vaginalis* is the cause of 11% of all cases of nongonococcal urethritis in males (8).

Respiratory distress was found in a full-term, normal male infant with *T. vaginalis* who had severe respiratory difficulties after delivery. A wet preparation of thick, white sputum showed few leukocytes and motile flagellates, which were identified as *T. vaginalis*. This study supports previous data indicating that this organism may cause neonatal pneumonia (1, 8, 11).

Factors that influence the inflammatory response are not clearly defined but may include hormonal levels, coexisting vaginal flora, and the strain and concentration of the organisms present. Lymphocytes from patients with active trichomoniasis show a proliferative response when incubated in the presence of secretory and cellular products of *T. vaginalis*. This finding indicates that delayed hypersensitivity reactions may act to modify inflammatory responses.

Several mechanisms are used by *T. vaginalis* to maintain the infection in the female urogenital tract. Recognition and binding to vaginal epithelial cells by adhesins that resemble metabolic enzymes are the first step (Table 25.2). Immune responses are evaded by masking the organisms with host proteins, shedding of trichomonad proteins into the secretions, and degradation of all immunoglobulin subclasses and complement (8). Parasites undergo phenotypic variation between surface and cytoplasmic expression of a number of immunogenic proteins against which host antibody is directed. Phenotypic variation occurs in response to iron-regulated proteins, virus-induced and repressed proteins, erythrocyte binding proteins and hemolysins, proteinases, and adhesins and contributes to the organism's overall antigenic diversity and complexity (12). Subpopulations of

TABLE 25.2 Virulence factors associated with *Trichomonas vaginalis* pathogenesis[a]

Capability	Virulence factor(s)
Nutrient acquisition and adherence	Receptors for nutrient acquisition Adhesin that is time, temperature, pH dependent Adhesin proteins, which are receptors to host extracellular matrix proteins Carbohydrates, which provide basis for ligand-receptor binding Presence of cysteine proteinases required Microfilaments concentrated on side of parasite that attaches Laminin may be target for adhesion
Cysteine proteinases	Enzymes that are lytic factors in hemolysis of erythrocytes Required for adherence to epithelial cells
Contact-independent mechanisms	Cell detaching factor (CDF) causes cytopathic effects in cell culture (not seen in nonpathogen *Pentatrichomonas hominis*) CDF levels correlate with severity of clinical disease
Damage of target cell plasma membrane	Pores created in erythrocyte membranes
Interaction with vaginal flora	Normal vaginal pH 4.5; *T. vaginalis* thrives in less acidic pH (pH > 5) Rise in pH produces concomitant reduction or loss of protective lactobacilli
Immune system evasion	Surface coating with host proteins Shedding of immunogens Cysteine proteinase degradation of IgG and IgA and complement Epitope phenotypic variation Continuous secretion of large amounts of highly immunogenic soluble antigens short-circuits antibody response
Regulation of virulence genes	General nutrient limitations Environmental factors (iron) Parasite factors (double-stranded RNA virus)

[a] Adapted from references 1, 2, and 12.

trichomonads express high- or low-iron-regulated proteins depending on the iron status of microenvironments within the vagina. Thus, the expression of virulence genes is controlled by both parasite and host environmental factors. Populations of *T. vaginalis* from fresh clinical isolates probably comprise a heterogeneous population, thus guaranteeing that some organisms will be able to infect the host. Based on current knowledge, it appears that interference with trichomonad mucin receptors and proteinases may be a strategy to prevent colonization with this parasite.

Diagnosis

Unfortunately, clinical manifestations are not reliable criteria for the diagnosis of trichomoniasis; demonstration of the parasite is required for accurate diagnosis of infection. It was demonstrated in the early 1980s that if the classic features such as "strawberry cervix" and frothy discharge were used alone in the diagnosis of trichomoniasis, 88% of infected women would not be diagnosed and 29% of uninfected women would be incorrectly diagnosed as having trichomoniasis (8).

Wet Mounts. The identification of *T. vaginalis* is usually based on the examination of wet preparations of vaginal and urethral discharges and prostatic secretions. This examination must be performed within 10 to 20 min of sample collection, or the organisms lose motility and may be very difficult to see. Several specimens may have to be examined to detect the organisms, and the sensitivity of this technique may vary between 40% and >80%. The specimen should be diluted with a drop of saline and examined under low power with reduced illumination for the presence of actively moving organisms; urine sediment can be examined in the same way. As the jerky motility of the trophozoite diminishes, it may be possible to see the movement of the undulating membrane, particularly under high dry power. Specimens should never be refrigerated (Fig. 25.5).

Since the morphology of nonpathogenic *Pentatrichomonas hominis* from stool samples is very similar to that of pathogenic *T. vaginalis*, it is important to ensure that the specimen is not contaminated with fecal material.

Stained Smears. Diagnostic tests other than wet preparations, such as permanent stains and fluorescent stains, can also be used. Organisms may be difficult to recognize in permanent stains; however, if a dry smear is submitted to the laboratory, Giemsa or Papanicolaou stain can be used (Fig. 25.6 and 25.7). Chronic *Trichomonas* infections may cause atypical cellular changes that can be misinterpreted, particularly on the Papanicolaou smear. The organisms are routinely missed on Gram stains (Fig. 25.6). Unfortunately, *T. vaginalis* does not always appear in its typical pear-shaped form but instead may resemble polymorphonuclear leukocytes. The number of false-positive and false-negative results reported on the basis of stained smears strongly suggests that the results should be confirmed by observation of motile organisms either from the direct wet mount or from appropriate culture media (1).

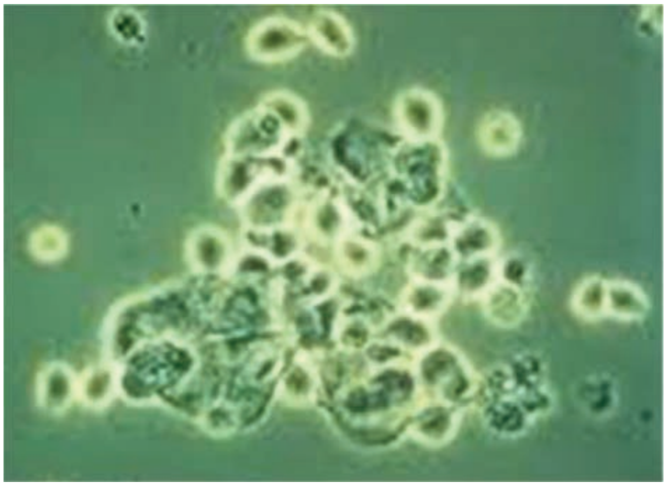


Figure 25.5 *Trichomonas vaginalis* wet mount (courtesy of the CDC Public Health Image Library).
doi:10.1128/9781555819002.ch25.f5

Culture. If culture techniques are used, it is mandatory that the specimen be collected correctly, immediately inoculated into the proper medium, and properly incubated. If these requirements are not met, the findings may represent a false-negative result. Although culture is more sensitive than wet mounts, this approach is not routinely used because of cost and inconvenience. To improve these parameters, a more convenient plastic envelope method is being widely used (13) (Fig. 25.8). This envelope approach allows both immediate examination and culture in one self-contained system. The system is commercially available as the InPouch TV (BIOMED Diagnostics, San Jose, CA), which serves as the specimen transport container, the growth chamber during incubation, and the "slide" during microscopy. Once it is inoculated, it requires no opening for examination, and positive growth occurs within 5 days. The sensitivity of this system is reported to be superior to that of other available culture methods (1, 8, 13). Also, no detectable differences were seen with the timing of the inoculation of the top or bottom pouch (13). This delayed-inoculation technique allows the initial reading of the wet mount and then inoculation of the culture pouch if the wet mount is negative. The InPouch TV test is the gold standard for clinical culture for *T. vaginalis* screening. The

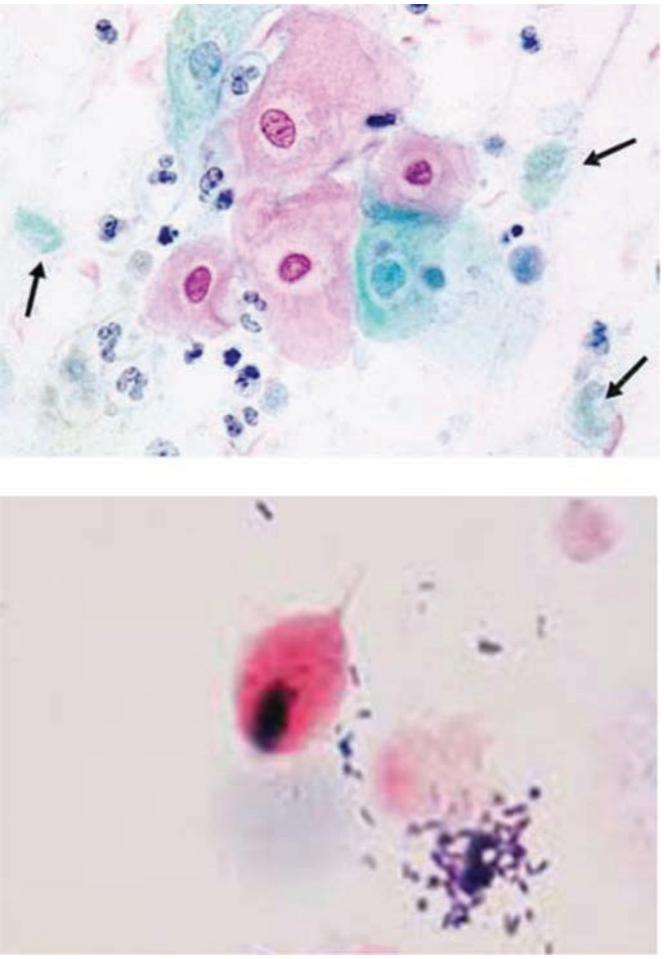


Figure 25.6 *Trichomonas vaginalis.* (Upper) Papanicolaou stain; (lower) Gram stain. doi:10.1128/9781555819002.ch25.f6

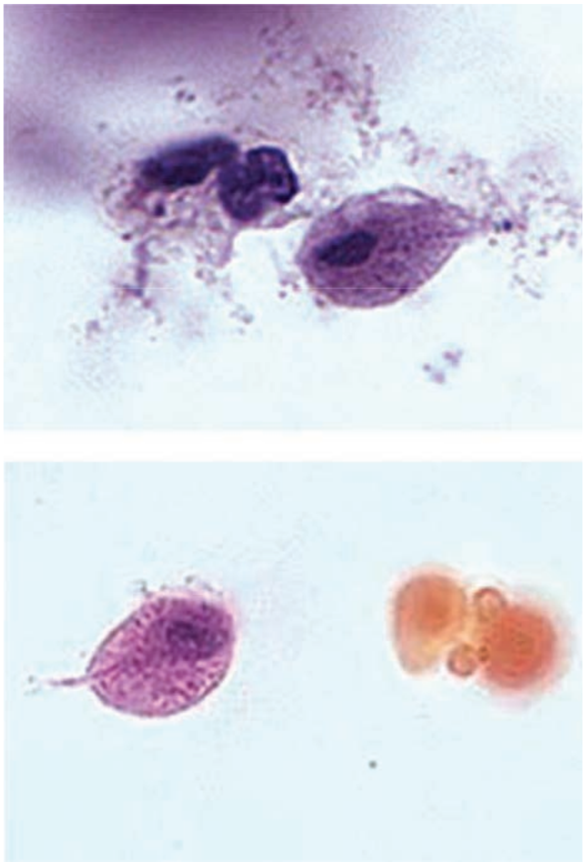


Figure 25.7 *Trichomonas vaginalis.* Giemsa stains of vaginal preparations (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch25.f7

current package insert recommends an examination period of 3 days. In a review of 2,499 InPouch tests spanning 13 years, the authors observed that examination up to 3 days will detect only 82.8% (95% confidence interval [CI], 79.0 to 86.2%) of positive specimens (14).

Various studies have been published regarding newer medium formulations and comparisons, including the use of modified Columbia agar (15). Although the results of this study were excellent, the specimens were processed at bedside, an approach that is often not practical for specimen collection. Cell culture methods use a variety of cell lines to recover *T. vaginalis* from clinical specimens, and they include McCoy cells. This approach has been reported to detect the organism at a concentration as low as 3 organisms/ml (8). However, this approach is not as practical as other available culture options and is rarely used in the routine clinical laboratory setting. Similarities in the results of these various diagnostic methods, particularly culture methods, have to be reviewed within the context of delivery systems and compliance with the proper use of transport options.

Antigen Detection. Screening patients for the presence of *T. vaginalis* has been somewhat limited by the available tests, many of which tend to be insensitive or expensive or require delays before the result is reported. A rapid diagnostic test for *T. vaginalis* infection has been developed and has proven to be much more sensitive than the wet-mount examination (Table 25.3). When compared with culture (InPouch), the XenoStrip-Tv (Xenotope Diagnostics, Inc., San Antonio, TX) was 76.7% sensitive and 99.8% specific and 79.4% sensitive and 97.1% specific in two different studies (16). Rapid test performance did vary with the day of the culture-positive result, with a 71% decline in XenoStrip sensitivity for every additional day of delay until cultures were positive. This product is easy to

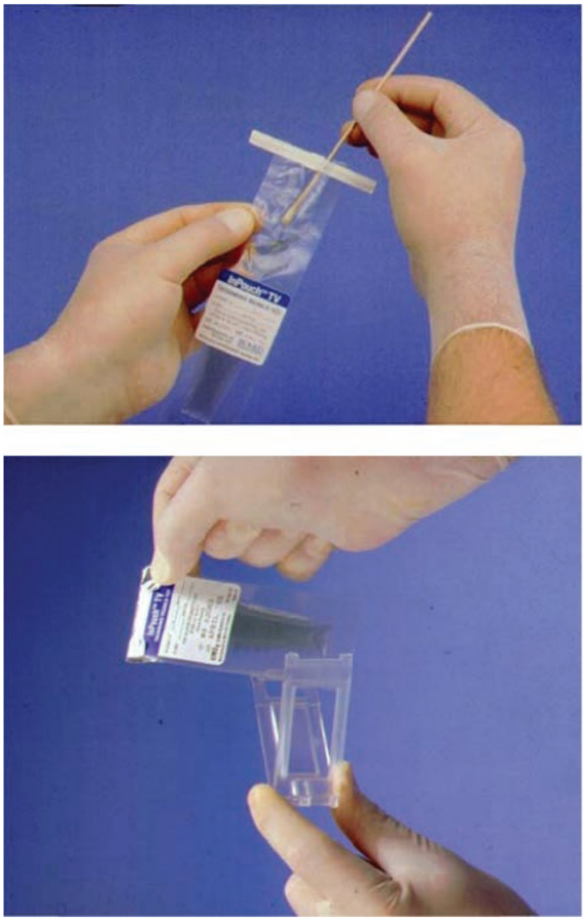


Figure 25.8 InPouch TV diagnostic system for culturing *Trichomonas vaginalis*. (Upper) The swab containing a specimen from the patient is being inserted into the liquid medium within the plastic pouch; (lower) the pouch is inserted into the plastic holder. (Photographs courtesy of BIOMED Diagnostics.) See also chapter 32. doi:10.1128/9781555819002.ch25.f8

use, is more sensitive than the wet mount, and should be considered as an alternative to the wet mount, particularly for point-of-care diagnosis when microscopy may be impractical (17).

Another rapid detection system for *T. vaginalis* uses an immunochromatographic capillary-flow (dipstick) assay, which provides results in 10 min. Compared with wet mount and culture, this system, OSOM Trichomonas Rapid Test (Genzyme Diagnostics, Cambridge, MA), showed 83.3% sensitivity and 98.8% specificity, while the wet mount has a sensitivity of 71.4% and a specificity of 100% (Fig. 25.9). This test also detected *T. vaginalis* in samples that required 48 to 72 h of incubation before becoming culture positive (18, 19). Compared to culture alone, OSOM was 83.5% sensitive and 99.0% specific.

Antibody Detection. Various techniques have been used to demonstrate antibodies against *T. vaginalis*; they include agglutination, complement fixation, indirect hemagglutination, gel diffusion, fluorescent-antibody assay, and enzyme-linked immunosorbent assay (ELISA). However, there are a number of reasons why this approach has not been used routinely. In some cases, an antibody response is not seen, due to test sensitivity or the timing of the test procedure. Also, antitrichomonal antibodies persist for long periods after therapy, and so current and past infections cannot be differentiated.

Molecular Testing. The use of PCR methods has led to improvements in *T. vaginalis* detection; nonviable organisms and target sequences can also be detected. In addition to various culture and transport options, there are currently several other products available, including the Affirm VPIII probe (Becton Dickinson, Cockeysville, MD), the Quik-Tri/Can latex agglutination test (PanBio Integrated Diagnostics, Baltimore, MD), and the T.VAG DFA (Light Diagnostics, Temecula, CA). The Affirm VP system uses synthetic oligonucleotide probes for the detection of both *Gardnerella vaginalis* and *T. vaginalis* from a single vaginal swab (8). A PCR-based ELISA with urine as the clinical specimen gave sensitivity and specificity data (compared with wet mount or culture) as 90.8% and 93.4%, respectively, in women and 92.7% and 88.6%, respectively, in men (21, 22). For clinical settings in which vaginal specimens are not available and culture is not an option, urine-based PCR-ELISA may be another option. In some settings, self-obtained vaginal swabs may be useful for PCR, and it appears that PCR may be more sensitive than culture for the diagnosis of *T. vaginalis* in males (1). Based on your patient population, client base, number of requests, and cost, these tests may be relevant for your laboratory test menu.

The results of the GenProbe Aptima *T. vaginalis* assay were compared with the Affirm VPIII *T. vaginalis* test. In the absence of an independent reference standard for trichomonal vaginitis, a positive result in either assay was deemed to represent true infection. The diagnostic accuracy of the combined NAA-based test construct was approximately 20 to 25% higher than that of the Affirm VPIII when modeled in populations with various prevalences of infectious vaginitis (22).

A battery of highly sensitive and specific PCR assays has been developed that detects *T. vaginalis* in urine. A protocol for differentiating among human *Trichomonas* species was also developed; these procedures could also be used for the validation of commercial nucleic acid amplification tests (23).

KEY POINTS—LABORATORY DIAGNOSIS

Trichomonas vaginalis

1. Wet-preparation (motile organisms) examination is recommended; the specimen should be examined immediately. If this is not possible, other methods should be considered.
2. Fluorescent stains and culture have also been recommended.
3. The newer rapid methods may not be as sensitive as culture or PCR, but organisms can be detected, often 48 to 72 h prior to cultures becoming positive for *T. vaginalis*.
4. If dry smears are submitted to the laboratory, they can be fixed with absolute methanol, allowed to dry, and stained like a thin blood smear (Giemsa stain).
5. Serologic tests are not routinely available.

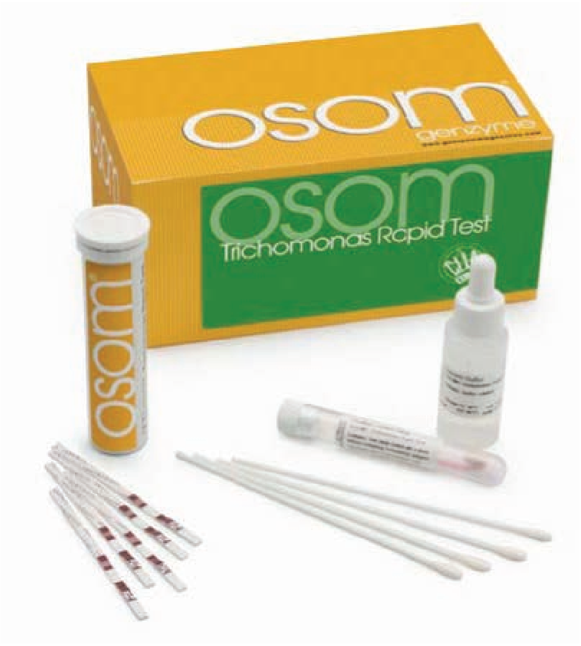


Figure 25.9 *Trichomonas vaginalis*. Direct antigen detection strip used for rapid testing (OSOM Trichomonas Rapid Test; courtesy of Genzyme Diagnostics, Cambridge, MA). doi:10.1128/9781555819002.ch25.f9

Treatment

Metronidazole is recommended for the treatment of urogenital trichomoniasis, although resistance to both metronidazole and other 5-nitroimidazoles has been reported (24). It is also recommended that all sexual partners of the patient be treated simultaneously to avoid immediate reinfection. Metronidazole-resistant *T. vaginalis* has been implicated in an increased number of cases; unfortunately, metronidazole is currently the only drug approved for the treatment of trichomoniasis in the United States (11). Although vaginal metronidazole creams and pessaries are available, their cure rate compared with oral drug administration is poor. Metronidazole can be taken as single or multiple doses, as well as administered intravenously. Additional information on therapy can be found in chapter 36.

One review reported that metronidazole reduced the risk of persistent infection in women with *T. vaginalis* infection but increased the incidence of preterm birth (25).

TABLE 25.3 Summary of commercially available kits for immunodetection of *Trichomonas vaginalis*

Diagnostic kit	Manufacturer and/or distributor[a]	Type of test	Sensitivity (%), specificity (%)	Comments
Affirm VPIII	Becton-Dickinson	DNA probe	93, 99	*Trichomonas vaginalis*, *Gardnerella vaginalis*, *Candida* spp.
Aptima *Trichomonas vaginalis* (ATV)	Hologic	Nucleic acid amplification test (NAAT)	94–100, 96–100	*Trichomonas vaginalis* (vaginal swab, endocervical swab, PreservCyt solution)
Quik-Trich	PanBio Integrated Diagnostics	Latex agglutination	95, 99	*Trichomonas vaginalis*
OSOM Trichomonas Rapid Test	Genzyme Diagnostics	Dipstick	83.3, 98.8	*Trichomonas vaginalis*; *T. vaginalis* detected in samples that required 48 to 72 h of incubation prior to becoming positive
Trichomonas vaginalis DFA[b] kit	Light Diagnostics—Chemicon	DFA[b]	97, 99	96.1% agreement with wet mount, but more positives found with DFA
XenoStrip-Tv	Xenotope Diagnostics	Dipstick	76.7, 99.8	*Trichomonas vaginalis*

[a] Becton Dickinson Diagnostic Systems, 7 Loveton Circle, Sparks, MD 21152; Chemicon International, Inc., 28835 Single Oak Drive, Temecula, CA 92590; Genzyme Diagnostics, One Kendall Square, Cambridge, MA 02139; Hologic, 35 Crosby Dr., Bedford, MA 01730; PanBio Integrated Diagnostics, 1756 Sulfur Springs Road, Baltimore, MD 21227; Xenotope Diagnostics, Inc., 3463 Magic Drive #350, San Antonio, TX 78229.

[b] DFA, direct fluorescent antibody.

Neonatal infection depends on maternal estrogen levels, which wane after the 3rd to 6th week of life; after this time, the infection may disappear. However, if required, treatment of neonates should be postponed until the 6th to 8th week of age and then given if the infant is symptomatic (8).

Although the cure rate is excellent, treatment failure can be due to several factors including noncompliance and reinfection. Published case reports of *T. vaginalis* strains that are clinically resistant to metronidazole are increasing; in the late 1990s, it was estimated that approximately 5 to 10% of all patient isolates demonstrated some level of resistance to metronidazole (26). Resistance can be linked to progressive decrease and eventual loss of the pyruvate: ferredoxin oxidoreductase, so that the drug-activating process is averted. The development of resistance in *T. vaginalis* is also accompanied by decreased expression of ferredoxin (11). Refractory cases are defined as cases in which two standard courses of therapy fail to cure the patient, and these cases can be very difficult to treat. Refractory cases are treated with higher (often double) doses of metronidazole for an extended period; this is effective approximately 80% of the time. Treatment protocols are now available for marginal, low, moderate, and high levels of metronidazole resistance. Therefore, the need for metronidazole susceptibility testing has become more important for clinically relevant patient care. Different methods are used as pharmacological screening procedures for new drugs, including simple cell enumeration. However, this approach is highly subjective. Another approach, which involves determination of acid phosphatase activity as the screening procedure, has been recommended. Compared with microscopy procedures, there are some clear advantages to these newer colorimetric methods, including greater speed and efficiency and less subjectivity (27). Other metabolic routes of *T. vaginalis* have also been used to develop susceptibility testing methods for determining antitrichomonal activity (27).

Demographic data and *T. vaginalis* isolates were collected from women attending six STI clinics participating in the STD Surveillance Network (SSuN). SSuN is a sentinel site surveillance network which, through the implementation of common protocols for collecting, reporting, and analyzing enhanced surveillance data, aims to improve the capacity of national, state, and local STI programs to detect, monitor, and respond rapidly to trends in STIs. *T. vaginalis* specimens from women undergoing physical examinations were systematically collected, either consecutively or on selected days of the week, during 2009 and 2010. A low prevalence of *in vitro* metronidazole resistance was seen. Minimum lethal concentrations of tinidazole were lower than those of metronidazole, and no tinidazole resistance was detected (28).

Epidemiology and Prevention

Trichomoniasis has been found in every continent and climate, with no season variability. It has a cosmopolitan distribution within all racial groups and throughout all socioeconomic levels. The annual incidence of trichomoniasis is estimated to be more than 275 million cases worldwide. The incidence can exceed 50% in patients attending STI clinics and depends on age, sexual activity, number of sexual partners, other STIs, sexual customs, phase of the menstrual cycle, and sensitivity of patient examination and specimen collection and testing.

Humans are the only natural host for *T. vaginalis*. Risk factors for trichomoniasis have also been investigated. The associations between HIV and trichomoniasis may relate to increased shedding of HIV due to local inflammation produced by *T. vaginalis*, increased susceptibility to HIV as a result of breaks in the mucosal barriers, a higher prevalence of STIs in HIV-infected individuals, and/or an increased susceptibility to HIV due to immunosuppression. Transmission of HIV is enhanced by coinfection with *T. vaginalis*, and treatment of trichomoniasis reduces the levels of HIV RNA (1).

A symbiotic relationship between *T. vaginalis* and *Mycoplasma hominis* has been documented; such a symbiosis may indicate a potential role of *T. vaginalis* in the transmission of bacterial infection (29). These findings suggest that this relationship between the two organisms may provide the bacteria with the ability to resist the host defense mechanisms and therapy.

Infection is acquired primarily through sexual intercourse, hence the need to diagnose and treat asymptomatic males. The organism can survive for some time in a moist environment such as damp towels and underclothes; however, this mode of transmission is thought to be very rare. Evidence implicating *T. vaginalis* as a potential contributor to poor outcomes in both women and men continues to accumulate. In women, this infection may play a role in the development of cervical neoplasia, postoperative infections, and potential problems with pregnancy. It is also seen as a factor in atypical pelvic inflammatory disease and infertility. In men, trichomoniasis causes nongonoccocal urethritis and contributes to male infertility. The importance of this infection needs to be recognized beyond the limits of the STI clinics (10, 30).

Vaccines. As with many STIs, infection with *T. vaginalis* does not produce long-term immunity. Although the antibody response to this organism is well documented, the antibodies provide only limited protection, and antibody titers disappear after therapy. Vaccine development has shown some promise; potential targets include a 100-kDa protein that is immunogenic across a number of *T. vaginalis* isolates, as well as essential adherence molecules such as adhesins, mucinases, and cysteine proteinases (11). Although several vaccine trials were held during the 1960s and the late 1970s, subsequent studies led to doubts about the efficacy of the vaccines. Later studies using a murine model have shown promise, and a bovine *Trichomonas foetus* model in cattle has also yielded promising results. A whole-cell *Trichomonas foetus* vaccine is now commercially available for cattle and provides protection, as well as accelerating the eradication of infection.

Summary. *T. vaginalis* is one of the neglected parasitic infections designated by the Centers for Disease Control and Prevention (CDC) which have been targeted as priorities for public health action. With the introduction of newer diagnostic testing, more attention can be devoted to surveillance, prevention, and treatment. Because of the increasing burden of trichomoniasis in certain obstetric populations, the question of whether screening and treatment during pregnancy improve perinatal outcomes should be readdressed. In addition, the association with pelvic inflammatory disease and HIV acquisition is troubling. No longer can we afford to continue to disregard this destructive parasite by not screening at-risk patients.

Toxoplasma gondii

Toxoplasma gondii is worldwide in distribution, is closely related to other coccidia, and also has certain similarities to malarial parasites. The parasites were first discovered in 1908, by Charles Nicolle and Louis Manceaux at the Pasteur Institute, in the North African rodent called the gundi, hence the species name *gondii*. At the same time, Alfonso Splendore described the organism from laboratory rabbits at the Portuguese Hospital in São Paulo, Brazil. However, the complete life cycle was not determined until 1970 (31). Although serologic evidence indicates a high rate of human exposure to the organism, the disease itself is relatively rare. *T. gondii* can infect many vertebrates as well as humans, but the definitive host is the house cat and other members of the *Felidae* family.

Life Cycle and Morphology

The life cycle is shown in Fig. 25.10. Humans can acquire the infection in several ways, either by the accidental ingestion of oocysts shed in cat feces or by the ingestion of rare or raw meats, particularly pork, lamb, or venison. Transmission can also occur *in utero*, by transfusion, and during organ transplantation (32–34). Toxoplasmosis should be considered as a possible cause of multiorgan failure after liver transplantation. The inclusion of serologic testing should be considered in routine donor testing. The most common means of infection is probably through ingestion of rare or raw meats. The oocysts shed in cat feces are 9 to 11 μm wide by 11 to 14 μm long and contain two sporocysts, each containing four sporozoites (Fig. 25.11). Cats shed oocysts after ingestion of any of the three infectious stages of *T. gondii*. Cats become infected from eating birds, small rodents, or other sources of raw meat. In the tachyzoite-induced life cycle of *T. gondii* in cats, the animals can shed up to 360 million oocysts per day and will shed for 4 to 6 days (34).

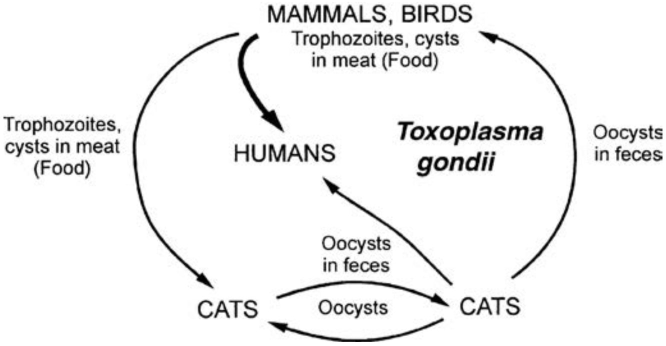


Figure 25.10 Life cycle of *Toxoplasma gondii*. The active forms (trophozoites) are also referred to as tachyzoites, while the resting stages within the cysts are called bradyzoites (very slow-growing trophozoites). doi:10.1128/9781555819002.ch25.f10

There are three infectious stages of *T. gondii*: the tachyzoites (in groups or clones), the bradyzoites (in tissue cysts), and the sporozoites (in oocysts). Tachyzoites rapidly multiply in any cell of the intermediate host (many other animals and humans) and in nonintestinal epithelial cells of the definitive host (cats). Bradyzoites are found within the tissue cysts and multiply very slowly; the cyst may contain few to hundreds of organisms, and intramuscular cysts may reach 100 μm in size. Although the tissue cysts may develop in visceral organs such as the lungs, liver, and kidneys, they are more prevalent in neural and muscular tissues, including the brain, eyes, and skeletal and cardiac muscle. Intact tissue cysts can persist for the life of the host and do not cause an inflammatory response (31).

The organisms are obligate intracellular parasites and are found in two forms in humans. The actively proliferating trophozoites or tachyzoites are usually seen in the early, more acute phases of the infection. The resting forms or cysts are found primarily in muscle and brain, probably as a result of the host immune response. The cysts contain the more slowly growing trophozoites or bradyzoites. Bradyzoites encyst approximately 8 to 10 days after entry into the host and differ from tachyzoites in being more resistant to pepsin, having a longer generation time, containing cytoplasmic vacuoles which may serve as carbohydrate stores, and being the only stage to initiate the enteroepithelial cycle and transform into oocysts in the feline intestine.

The trophozoites (tachyzoites) are crescent shaped and are 2 to 3 μm wide by 4 to 8 μm long (Fig. 25.12 and 25.13). One end tends to be more rounded than the other. Tachyzoites are the dissemination form. They are able to invade virtually all vertebrate cell types, where they multiply in a parasitophorous vacuole. Giemsa is the stain of

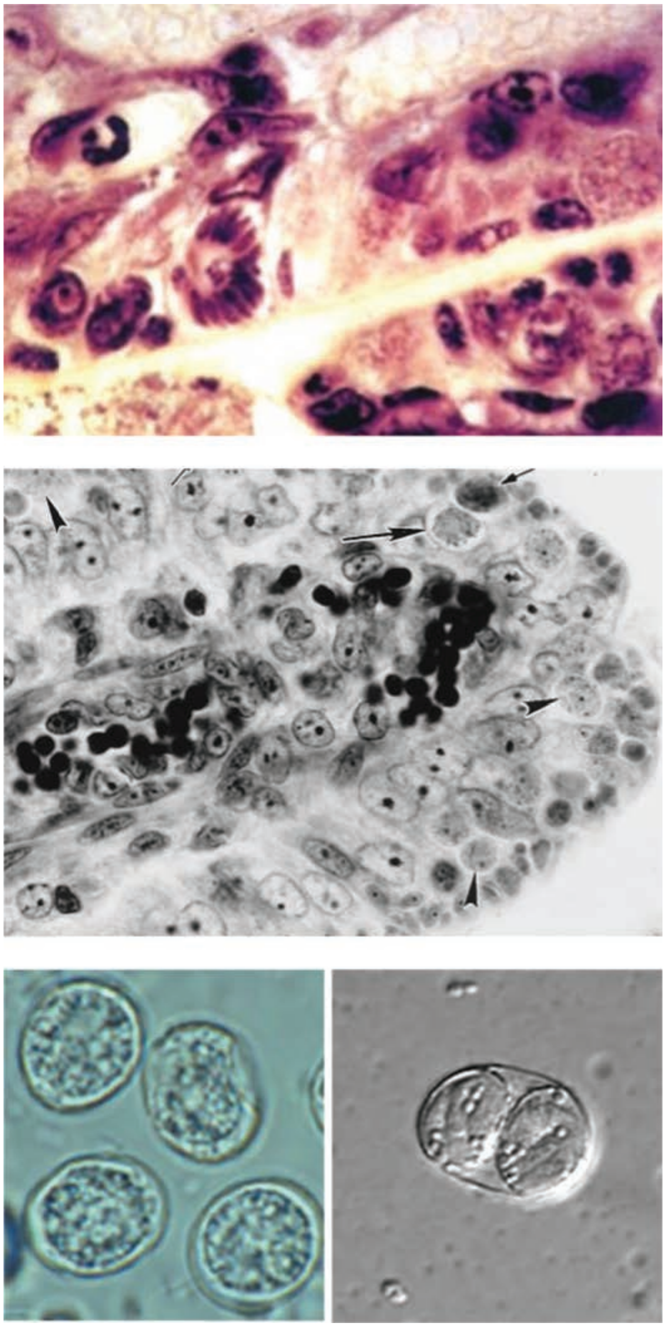


Figure 25.11 *Toxoplasma gondii*. (Top) Developmental stages in cat intestine. (Middle) Enteroepithelial stages of *T. gondii* in a small intestinal villus of a cat fed tissue cysts. Note the heavy parasitization of enterocytes, containing schizonts (type D) (small arrows) and male (large arrow) and female (arrowheads) gamonts. Hematoxylin and eosin stain (from reference 32, with permission). (Bottom, left) Unsporulated oocyst; (right) sporulated oocyst containing two sporocysts with sporozoites. (Courtesy of CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch25.f11

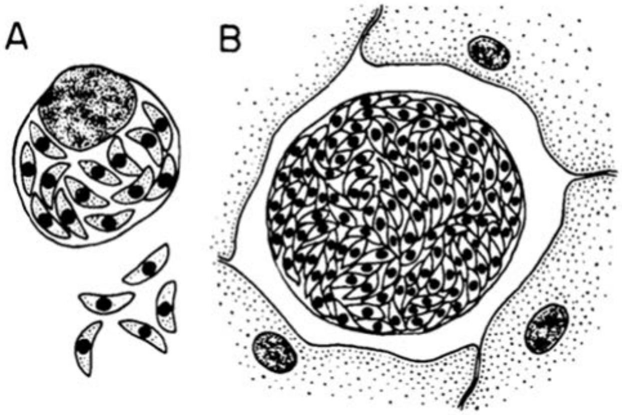


Figure 25.12 Forms of *Toxoplasma gondii* found in humans. (A) Trophozoites (tachyzoites) seen within a cell; (B) cyst containing the bradyzoites (illustration by Sharon Belkin; based on illustration from H. H. Najarian, *Textbook of Medical Parasitology*, The Williams & Wilkins Co., Baltimore, MD, 1967).
doi:10.1128/9781555819002.ch25.f12

choice; the cytoplasm stains pale blue and the nucleus stains red and is situated toward the broad end of the organism.

The cysts are formed in chronic infections, and the organisms within the cyst wall are strongly periodic acid-Schiff positive (Fig. 25.14). Bradyzoites result from the conversion of tachyzoites into a slow-dividing stage and form tissue cysts. These cysts are more or less spheroid in brain cells or elongated in muscular cells. During the acute phase, there may be groups of tachyzoites that appear to be cysts; however, they are not strongly periodic acid-Schiff positive and have been termed pseudocysts.

The cycle occurring in cats includes asexual multiplication and sexual reproduction in the small intestinal epithelial cells (31, 34). Sexual reproduction occurs only in felids (domestic and wild cats). The final stage of the reproductive cycle is the oocyst, which is passed in the feces. The shedding of oocysts begins 3 to 7 days after the ingestion of tissue cysts and may continue for up to 20 days. Infected cats can shed more than 100 million oocysts in their feces. They can infect a wide range of intermediate hosts—virtually all warm-blooded animals, from mammals to birds—when ingested with food or water. Oocysts are also infective for cats, although less efficiently. After defecation, development of infective sporozoites inside the oocysts requires 1 to 5 days and depends on environmental conditions. The developmental cycle in the cat takes approximately 19 to 48 days after infection with the oocysts but only 3 to 10 days after the ingestion of meat infected with cysts, e.g., a mouse.

The number of cats in the United States is estimated at around 70 million, with the number of feral cats probably being greater than that of domestic cats. Of the cats

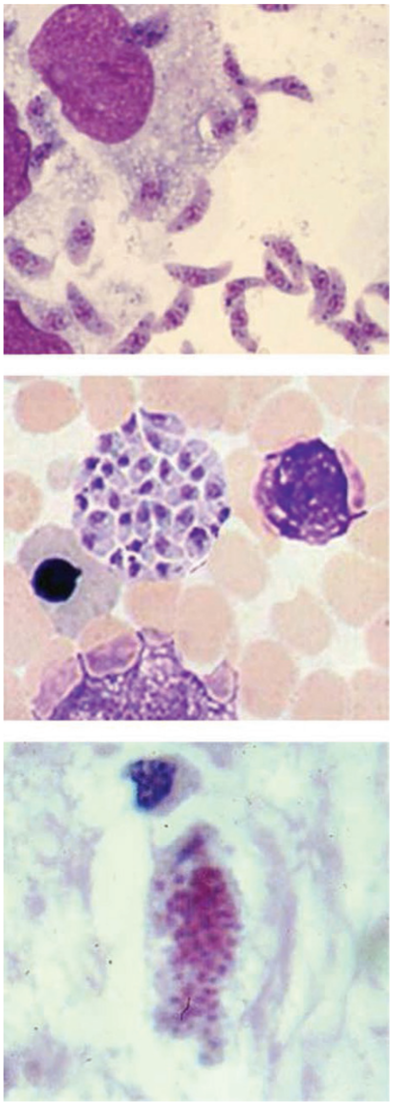


Figure 25.13 *Toxoplasma gondii*. (Top) Tachyzoites from mouse peritoneal fluid; (middle) tachyzoites seen within human bone marrow; (bottom) bradyzoites seen in human tissue. doi:10.1128/9781555819002.ch25.f13

examined within the United States, 1% had *T. gondii* oocysts in their feces. However, this may be because the period of shedding is usually only 1 to 2 weeks during the life of the cat and the oocysts are quite small, measuring approximately 10 µm. Consequently, the use of routine fecal examinations to determine whether cats are positive for *T. gondii* is somewhat limited and is not particularly helpful in determining whether the animal is shedding oocysts.

Clinical Disease

Approximately 25 to 30% of the world's human population is infected by *Toxoplasma*. Actually, the prevalences vary widely between countries and often within a given country or between different communities in the same region. Low seroprevalences (10 to 30%) have been observed in North America, in Southeast Asia, in northern Europe, and in Sahelian countries of Africa. Moderate prevalences (30 to 50%) have been found in countries of central and southern Europe, and high prevalences have been found in Latin America and in tropical African countries (35).

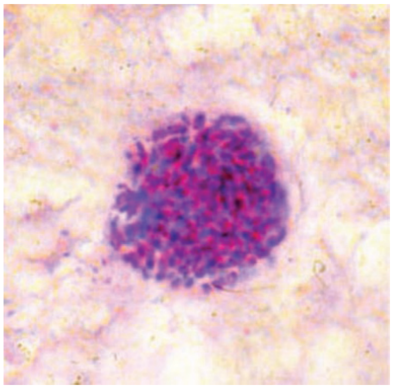


Figure 25.14 *Toxoplasma gondii*. Tissue cyst, PAS stain. Each cyst contains 50 to 5,000 bradyzoites, which replicate following cyst rupture, unless destroyed by cellular immunity. doi:10.1128/9781555819002.ch25.f14

When the tachyzoites are actively proliferating, they invade cells adjacent to the original infected cell as it ruptures. This process creates continually expanding focal lesions. Once the cysts are formed, the process becomes quiescent, with little or no multiplication and spread. In the immunocompromised or immunodeficient patient, a cyst rupture or primary exposure to the organisms often leads to lesions. The organisms can be disseminated via the lymphatics and the bloodstream to other tissues. Disintegration of cysts may give rise to clinical encephalitis in the presence of apparently adequate immunity.

The large number of people who are serologically positive for *T. gondii* suggests that the majority of infections are benign, with most people exhibiting few (e.g., cold or light case of the flu) or no symptoms. The most severe symptoms are seen with congenital, transplacental infections or infections in compromised patients (Tables 25.4

TABLE 25.4 People at risk for severe toxoplasmosis

Category	Comments
Infants born to mothers who are first exposed to *Toxoplasma* infection several months before or during pregnancy	Mothers who are first exposed to *Toxoplasma* more than 6 months before becoming pregnant are not likely to pass the infection to their children.
Persons with severely weakened immune systems	Infection that occurred at any time during life can reactivate.

TABLE 25.5 Toxoplasmsosis: mechanisms of human infection

Mechanism	Comments
Infection through cysts	Any meat from warm-blooded animals and birds (lamb, pork, beef, chicken, etc.)
Cyst resistance	Cysts remain infectious in refrigerated carcasses (1–6°C) or minced meat for up to 3 weeks; freezing at –12°C or lower for at least 3 days is usually acceptable, although it depends on the thickness of the meat
Solid organ transplantation	Infection transmitted through a cyst-containing donor organ; heart transplants higher risk than liver, lung, or kidney patients
Infection through oocysts	Risk of infection in cats increases when weather is warm and moist; single cat can shed >100 million oocysts, which require 1–5 days to sporulate and become infective; direct cat contact not considered major risk; oocysts may remain infective in cat litter up to 14 days; once sporulated can survive viable for more than 1 yr
Contamination of water	Remain viable for long periods and can resist freezing and moderately high water temperatures; not killed by routine chemical/physical treatments currently used by water treatment plants; freshwater runoff may contaminate seawater; viable oocysts found in various species of shellfish (oysters, clams, mussels)
Contamination of soil, vegetables, fruits	Contact with soil; particularly high risk in children
Infection through tachyzoites	Fragile stage outside of the host; not as likely as other means of infection, but linked to drinking of unpasteurized goat's milk
Congenital infection	Tachyzoites colonize placental tissues and gain access to the fetus in about 30% of cases; decreasing seroprevalence of toxoplasmosis in industrialized countries may have consequences for the risk of infection during pregnancy
Infection through injection	Rare, but possible via needlestick injuries or scratching while manipulating tachyzoites from the RH strain[a]; blood transfusion and/or bone marrow possible

[a] The RH type I strain has been extensively used for defining the biology of *Toxoplasma*, ranging from active invasion and replication to host cell entry, and for molecular genetic analyses. RH was initially isolated from a 1939 case of toxoplasmic encephalitis by Albert Sabin and was subsequently cloned by Elmer Pfefferkorn for *in vitro* culture in 1977.

and 25.5). Toxoplasmosis can be categorized into four groups: (i) disease acquired in immunocompetent patients, (ii) disease acquired or reactivated in immunosuppressed or immunodeficient patients, (iii) congenital disease, and (iv) ocular disease.

Acquired Infections in Immunocompetent Individuals. In approximately 90% of cases, no clinical symptoms are seen during the acute infection. However, 10 to 20% of patients with acute infection may develop painless cervical lymphadenopathy. This presentation is benign and self-limited, with symptoms resolving within weeks to months. Acute visceral manifestations are seen in rare cases. In one study from French Guiana over a period of 6.5 years, 13 of 16 patients had consumed game in the 2 weeks prior to the onset of symptoms and in eight cases the game was undercooked (36). Although other cases have been reported during the last decade, very few were reported in detail. In reviewing the possible link between *T. gondii* and schizophrenia, it appears that some cases of acute toxoplasmosis in adults are associated with psychiatric symptoms such as delusions and hallucinations (37).

Infections in Immunocompromised Patients. Infections in immunocompromised patients can lead to severe complications; underlying conditions that may influence the course of the disease include various malignancies (such as Hodgkin's disease, non-Hodgkin's lymphomas, leukemias, and solid tumors), collagen vascular disease, organ transplantation, and AIDS. In immunocompromised patients, the central nervous system (CNS) is primarily involved, with diffuse encephalopathy, meningoencephalitis, or cerebral mass lesions. More than 50% of these patients show an altered mental state, motor impairment, seizures, abnormal reflexes, and other neurologic sequelae. Studies show that even in these groups, most patients receiving chemotherapy for toxoplasmosis will improve significantly or have complete remission. However, in those with AIDS, therapy must be continued for long periods to maintain a clinical response.

In genotyping studies, it appears that the type of *T. gondii* strain does not predominantly influence the pathogenesis of toxoplasmosis in immunocompromised patients. Thus, regardless of the strain genotype, there remains the need for specific prophylaxis in these patients infected by *T. gondii* (38).

Transplant recipients. In transplant recipients, disease severity depends on previous exposure to *T. gondii* by the donor and recipient, the type of organ transplanted, and the level of immunosuppression of the patient. Disease can be due to reactivation of a latent infection or an acute primary infection acquired directly from the transplanted organ (39). Stem cell transplant recipients are particularly susceptible to severe toxoplasmosis, primarily due to reactivation of a previously acquired latent infection. If stem cell transplant recipients have a positive serologic test prior to transplantation, they are known to be at risk for severe disseminated toxoplasmosis (40). All potential transplant recipients should be tested for *Toxoplasma*-specific IgG antibodies to determine their antibody status. An individual with acute acquired infection often produces detectable IgG and IgM antibodies, while those with reactivation may or may not have an increase in the levels of IgG antibodies and normally do not demonstrate an IgM response. Seronegative cardiac transplant recipients who receive an organ from a seropositive donor may develop toxoplasmic myocarditis; this disease presentation may also mimic organ rejection.

AIDS patients. Patients who become infected with *T. gondii* risk developing disease when their CD4$^+$ T-lymphocyte count falls below 100,000/ml. Fever and malaise usually precede the first neurologic symptoms; headache, confusion, seizures, or other focal signs strongly suggest the diagnosis of toxoplasmosis.

Toxoplasma encephalitis (TE) was reported as a life-threatening opportunistic infection among patients with AIDS prior to the use of highly active antiretroviral therapies. This condition is fatal if untreated. Most AIDS patients with TE have detectable IgG antibodies to *T. gondii*; however, <5% may not have antibody in their serum. Psychiatric manifestations of *T. gondii* are also seen in immunocompromised individuals with AIDS in whom latent infections have become reactivated. Altered mental status occurs in approximately 60% of patients, with symptoms including delusions, auditory hallucinations, and thought disorders (37). A characteristic imaging finding of toxoplasmosis is the "asymmetric target sign." Typically, any abscess forms a ring-enhancing lesion; however, in toxoplasmosis, there is an enhancing, eccentrically situated nodule within this ring. This sign can be seen by postcontrast computed tomography and magnetic resonance imaging and is virtually pathognomonic (Fig. 25.15). Unfortunately, it is only present in approximately one-third of patients, and in the remaining patients *Toxoplasma* lesions within the brain have a variable appearance depending on the degree of immunosuppression.

Disseminated toxoplasmosis should be considered in the differential diagnosis of immunocompromised patients with culture-negative sepsis, particularly if combined with neurologic, respiratory, or unexplained skin lesions. Examination of Wright's-stained peripheral blood smears or antitoxoplasma immunoperoxidase studies of skin biopsy specimens may be diagnostic (31).

In histologically confirmed cases of TE, when the majority of the patients received some therapy, the overall prognosis has been poor, with the median survival following initiation of therapy being 4 months. No beneficial or harmful effects could be attributed to the use of corticosteroids, and toxicity attributed to pyrimethamine-sulfonamide therapy was reported for many patients. A clinical relapse of TE has been seen in approximately half of the patients discharged from the hospital.

Although an inflammatory process is observed during TE in HIV-positive patients, the major cause of CNS lesions is probably uncontrolled parasite multiplication rather than immunopathologic changes. Also, *T. gondii* is capable of enhancing HIV-1 replication within reservoir host cells and, simultaneously, HIV-1 itself undermines acquired immunity to the parasite, promoting reactivation of chronic toxoplasmosis.

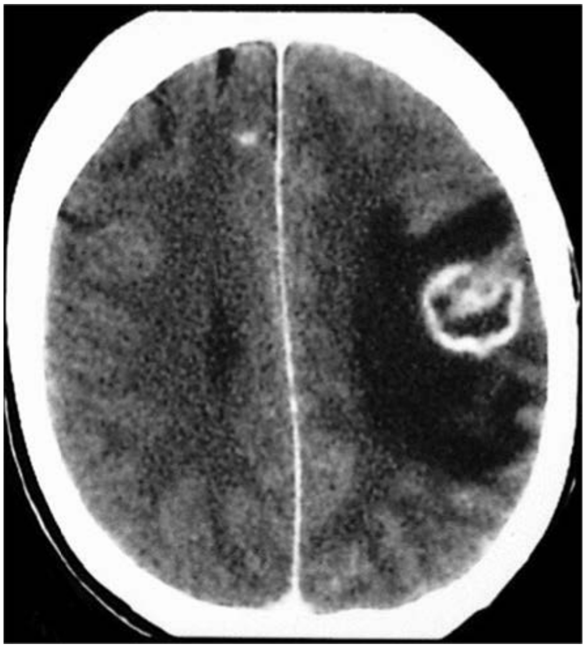


Figure 25.15 *Toxoplasma gondii.* CNS toxoplasmosis in patient with AIDS. Postinfusion computer tomography scan shows a moderately sized ring enhancing lesion with an eccentric nodule, which also enhanced. The corticomedullary location and marked surrounding edema are characteristic of toxoplasmosis. (Courtesy of P. Harrison, Vancouver; http://tmcr.usuhs.edu/tmcr/chapter45/imaging4.htm.) doi:10.1128/9781555819002.ch25.f15

Respiratory disease due to *T. gondii* has also been recognized in immunocompromised patients, and the few cases that have been found in HIV-positive patients have been in association with CNS disease. The finding of pulmonary toxoplasmosis in the absence of neurologic findings in HIV-positive patients emphasizes the importance of considering this organism as the cause of pulmonary disease in such patients with respiratory symptoms.

Congenital Infections. Congenital infections result from the transfer of parasites from the mother to the fetus when she acquires a primary infection during pregnancy. In 90% of patients, no clinical symptoms are apparent during acute infection. Congenital infections may be particularly severe if the mother acquires the infection during the first or second trimester of pregnancy. At birth or soon thereafter, symptoms in these infants may include retinochoroiditis, cerebral calcification, and occasionally hydrocephalus or microcephaly. Symptoms of congenital CNS involvement may not appear until several years later.

The risk of congenital toxoplasmosis depends on the timing of the mother's acute infection. Transmission to the fetus increases with gestation age: 15 to 25% in the first trimester, 30 to 45% in the second, and 60 to 65% in the third. However, the severity of congenital disease decreases with gestation age. Signs of infection at delivery are present in 21 to 28% of those infected in the second trimester and in ≤11% of those infected in the third trimester. Overall, 10% are born with severe disease. Congenital transmission can occur even if the mother is immune, although this is rare; reinfection of the mother during pregnancy is possible, particularly if she comes in contact with large numbers of infective cysts and/or oocysts.

The characteristic symptoms of hydrocephalus, cerebral calcifications, and chorioretinitis resulting in mental retardation, epilepsy, and impaired vision represent the most severe form of the disease. Cerebral lesions may calcify, providing retrospective signs of congenital infection.

In the case of seroconversion by the mother during pregnancy, prenatal diagnosis is usually performed by analysis of amniotic fluid. However, it is important to remember that despite the use of advanced diagnostic methods, some cases of congenital toxoplasmosis cannot be detected early. This demonstrates the importance of careful follow-up of newborns who are at risk. Treatment of the mother may reduce the incidence of congenital infection; therefore, rapid and accurate diagnosis should be emphasized.

Ocular Infections. Chorioretinitis in immunocompetent patients is generally due to an earlier congenital infection. Patients may be asymptomatic until the second or third decade; at that point, cysts may rupture, with lesions then developing in the eye. The number of people who develop chorioretinitis later in life is unknown but may represent over two-thirds of the total. Also, up to 30% of patients relapse after treatment. Chorioretinitis is usually bilateral in patients with congenitally acquired infection and is generally unilateral in patients with recently acquired infection.

Diagnosis

The diagnosis of *Toxoplasma* infection in immunocompetent individuals relies on serology. Serologic diagnosis is usually retrospective and is used to determine the immune status, such as (i) in a pregnant woman, preferably at an early stage of pregnancy; (ii) in a patient with uveitis or retinochoroiditis with no known history of congenital infection; or (iii) in a graft setting, i.e., in organ donors or transplant recipients. In patients with fever or lymphadenopathy, serologic testing allows the differential diagnosis of cytomegalovirus, Epstein-Barr virus, or HIV infections, as well as other diseases associated with lymphadenopathy (35).

Other procedures include PCR, examination of biopsy specimens, buffy coat cells, or cerebrospinal fluid, and isolation of the organism in tissue culture or in laboratory animals (Tables 25.6 and 25.7). It is difficult to demonstrate the parasite in lymph node biopsy specimens, and while some authorities think the histologic appearance is very characteristic, others consider the histologic changes to be nonspecific. However, because many individuals have been exposed to *T. gondii* and may have cysts within the tissues, recovery of organisms from tissue culture or animal inoculation may be misleading, since the organisms may be isolated but may not be the etiologic agent of disease. For this reason, serologic tests are often recommended as the diagnostic approach of choice. However, two representative situations in which the detection of organisms may be very significant are (i) tachyzoite-positive smears and/or tissue cultures inoculated from cerebrospinal fluid and (ii) in patients with acute pulmonary disease, the demonstration of tachyzoites in Giemsa-stained smears of bronchoalveolar lavage fluid, with some tachyzoites being extracellular and some being intracellular.

Emphasis on pediatric patients, particularly neonates, is seen in the extensive literature on serologic testing. An algorithm for the diagnosis and treatment of neonatal toxoplasmosis is seen in Fig. 25.16; a summary of risk management issues in pregnancy can be seen in Fig. 25.17. An understanding of the difficulties in test interpretation is helpful. First, all newborns from mothers who are antibody positive will have passively transferred maternal IgG. These titers can be quite high but may not indicate infection in the baby. The detection of IgM antibodies, which do not cross the placental barrier, provides a much more accurate indication of infection in the newborn. There can still be situations in which a false-negative (IgG saturation of antigen receptors) or false-positive (presence of rheumatoid factor and antinuclear antibody) result is possible. There is evidence to indicate that in certain instances, the presence of IgM antibody titers in single serum specimens cannot be used to indicate recent exposure, particularly since IgM can persist beyond 2 years (35, 39, 41). Interpretation of serologic tests for IgM can be seen in Fig. 25.18. A current means of confirming or ruling out a recent infection is the determination of IgG avidity, a method which relies on the increased affinity of the antibody for its target antigen during the course of natural immunity following infection.

In the United States, the first FDA-approved test (Vidas Toxo IgG avidity assay; bioMérieux) was approved in 2011. A high avidity ratio excludes an infection acquired in the preceding 4 months with most commercial tests, ruling out an infection acquired during gestation when the test is performed during the first trimester of pregnancy. In routine practice, avidity testing allows the avoidance of unnecessary spiramycin treatment in pregnant women and

TABLE 25.6 Clinical use of immunodiagnostic tests[a]

Questions to answer	Tests to use	Interpretation	Comments
Is the patient currently infected?	IgG assays	If IgG positive = infected If IgG negative, retest in 3 weeks if acute infection suspected	A single specimen is satisfactory to determine antibody status. Situations where baseline information is helpful: (i) women of childbearing age tested prior to conception; (ii) before initiation of immunosuppressive therapy; (iii) after determination of HIV-1-positive status; (iv) organ donors prior to transplantation.
Has the patient recently acquired the infection?	IgM and IgA assays	If IgG positive and IgM negative = infected for more than 6 months If IgG positive and IgM positive = infection within last 2 years, or false-positive IgM result	Specimens drawn 2 wk apart may or may not be helpful; often an increase in IgG or IgM titer is not possible; titers may already have reached a plateau when first sample is drawn.
How recently was the infection acquired by the patient?	IgG avidity, differential agglutination	If IgG avidity high = infected at least 12 wk earlier If IgG avidity low = recent infection possible; some people have persistent low IgG avidity for months after infection	If IgG avidity is low, draw second sample 3 weeks after first sample; send both specimens to *Toxoplasma* reference laboratory for confirmation of IgG, IgM, and IgG avidity results and possibly differential agglutination and IgA and IgE testing.
Is evidence of active infection present?	PCR, culture, antigen detection	*Toxoplasma* isolated from 95% of placentas of congenitally infected newborns when mother has not been treated and from approximately 81% when the mother has received therapy	Attempts should be made to isolate *T. gondii* from the placenta, amniotic fluid, and cord blood to confirm the diagnosis.

[a] Adapted from references 33 and 39.

unjustified long-term repeated follow-ups of fetuses and infants, provided that the test is performed before the fourth month of pregnancy (35). The ARCHITECT Toxo IgG assay (Abbott), using recombinant antigens, showed excellent specificity and sensitivity for acute phase as well as past infection specimens. The ARCHITECT Toxoplasmosis panel can be reliably used to rule out acute *T. gondii* infection in pregnant women (42).

The Sabin-Feldman dye test, one of the first methods used to diagnose toxoplasmosis, is based on the fact that in the presence of immune serum, *T. gondii* loses its affinity for methylene blue stain. Because this test uses live *Toxoplasma* trophozoites, however, most laboratories do not provide it routinely. A specific guide to the interpretation of serologic titers for toxoplasmosis is provided in Table 25.8. Interpretation of toxoplasmosis serology in pregnant women can be seen in Fig. 25.19.

The serologic diagnosis of toxoplasmosis is very complex and has been discussed extensively in the literature; a number of additional procedures, some of which are automated, have been developed and reported in the last few years (43). These methods include enzyme immunoassays, some of which are automated (20), ELISAs (39, 41, 44, 45), direct agglutination (39), an immunosorbent agglutination assay (39), an indirect immunofluorescence assay, immunocapture (39), and immunoblotting (46). Evaluations of various commercial assays have also been discussed (39, 47).

A great deal of molecular biology-based work involving PCR for the diagnosis of toxoplasmosis and strain typing has also been done (35, 40, 46, 48–51). This technology may be useful in situations where knowledge of recent infection is necessary. PCR has also been used to detect *T. gondii* in deparaffinized ocular tissue sections, cerebrospinal fluid, blood, and bronchoalveolar fluid; however, the absence of *Toxoplasma* DNA does not exclude toxoplasmosis. PCR can be very helpful in evaluating fetal infection; certainly the time needed to detect parasites is much shorter than when using mouse or tissue culture approaches. Sensitivity depends on the clinical presentation; it is higher when confirming congenital toxoplasmosis from amniotic fluid but lower in patients with infection reactivation such as HIV-positive patients.

TABLE 25.7 General comments on the performance and interpretation of serologic results for the diagnosis of toxoplasmosis[a]

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1. Specific IgM normally develops early, within 1–2 wk after primary infection.
2. Specific IgG normally develops within 4 wk after primary infection.
3. Production and increase in titers usually peak within 4–8 wk, but in individual cases the increase in IgM may continue for some weeks and IgG for several months.
4. The antibody result from a single serum sample gives no clear indication of when the infection occurred.
5. A rise in titer (IgG) should be demonstrated between 2 or more samples tested in the same test run; a 4-tube antibody increase is required to support the diagnosis of acute febrile toxoplasmosis. If serum is taken during late infection or for the presence of lymphadenopathy, titers in excess of 1:1,000 should be present.
6. The presence of IgM does not confirm a very recent infection; a positive IgM result has low predictive value for identifying primary *T. gondii* infection.
7. The amount of specific IgM may decrease to below the detection level in less than 3 mo after infection; however, IgM may be detectable for many months after the primary infection (can be detected as long as a year or more after primary infection). IgM antibody can persist for 6 mo in the conventional test but as long as 6 yr in the capture test.
8. IgG avidity gradually increases after primary infection. Low or equivocal avidity results can persist for months to >1 yr after primary infection. High-avidity test results seen in individuals infected for at least 3 to 5 mo.
9. For congenital infections, an IgG titer of >1:1,000 indicates a tentative diagnosis, pending confirmation of a positive IgM test, the exclusion of rheumatoid factor and antinuclear antibody, and the isolation of *Toxoplasma*, if possible. Passively transferred antibody shows a 10-fold decay every 3 mo; during infection, high antibody titers are stable or will increase.
10. Specific IgA results are not particularly helpful. A negative result does not exclude, nor does a positive result confirm, a recent primary infection.
11. Termination of pregnancy may be offered to women who seroconvert in the first 8 wk of pregnancy and to those infected in the first 22 wk of pregnancy when fetal infection is confirmed. A more conservative approach is based on abortion only if there is ultrasonographic evidence of hydrocephalus.
12. In the context of recurrent pregnancy loss, intravenous immunoglobulin therapy can cause confusion in the interpretation of serologic results for the diagnosis of toxoplasmosis; the appearance of anti-*Toxoplasma* antibodies may lead to the conclusion that seroconversion has occurred. Also, if positive serologic results are seen, the woman may be incorrectly considered immune.

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[a] Adapted from references 20, 35, 36, 39, 41, 44.

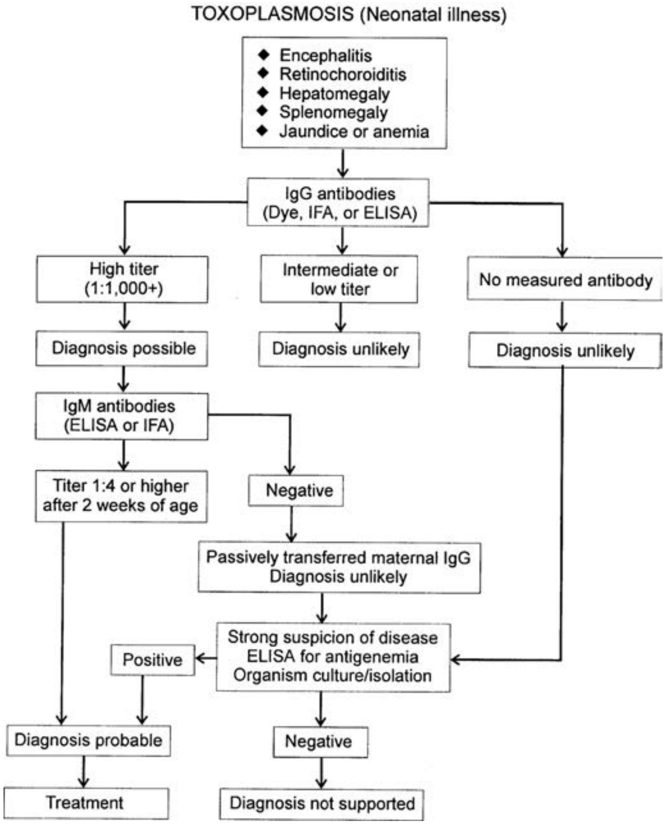

TOXOPLASMOSIS (Neonatal illness)
◆ Encephalitis
◆ Retinochoroiditis
◆ Hepatomegaly
◆ Splenomegaly
◆ Jaundice or anemia
IgG antibodies (Dye, IFA, or ELISA)
High titer (1:1,000+)
Intermediate or low titer
No measured antibody
Diagnosis possible
Diagnosis unlikely
Diagnosis unlikely
IgM antibodies (ELISA or IFA)
Titer 1:4 or higher after 2 weeks of age
Negative
Passively transferred maternal IgG Diagnosis unlikely
Strong suspicion of disease ELISA for antigenemia Organism culture/isolation
Positive
Diagnosis probable
Negative
Treatment
Diagnosis not supported

Figure 25.16 Chart for the diagnosis and treatment of neonatal toxoplasmosis. (Adapted from reference 39.) doi:10.1128/9781555819002.ch25.f16

Antigen detection has improved during the last few years; however, this approach still appears to lack sensitivity when used with human samples. Antigen detection may be helpful in diagnosing the compromised patient in whom antibody titers are low or absent. This serologic approach can also clarify low titers, i.e., those found in early acute infection or chronic infection. This test capability can also be very helpful in patients with monoclonal gammopathies, whose titers to *T. gondii* may be extremely high but may not be causing the clinical condition. See Algorithm 25.1.

KEY POINTS—LABORATORY DIAGNOSIS

Toxoplasma gondii

1. Serologic results are normally used to diagnose toxoplasmosis. Results of all other procedures must be interpreted in light of the serologic findings.
2. *T. gondii* identified in histologic preparations or isolated in animals or in tissue culture systems may or may not be the causative agent of the symptoms (many individuals harbor the organisms in their tissues but have no symptoms).

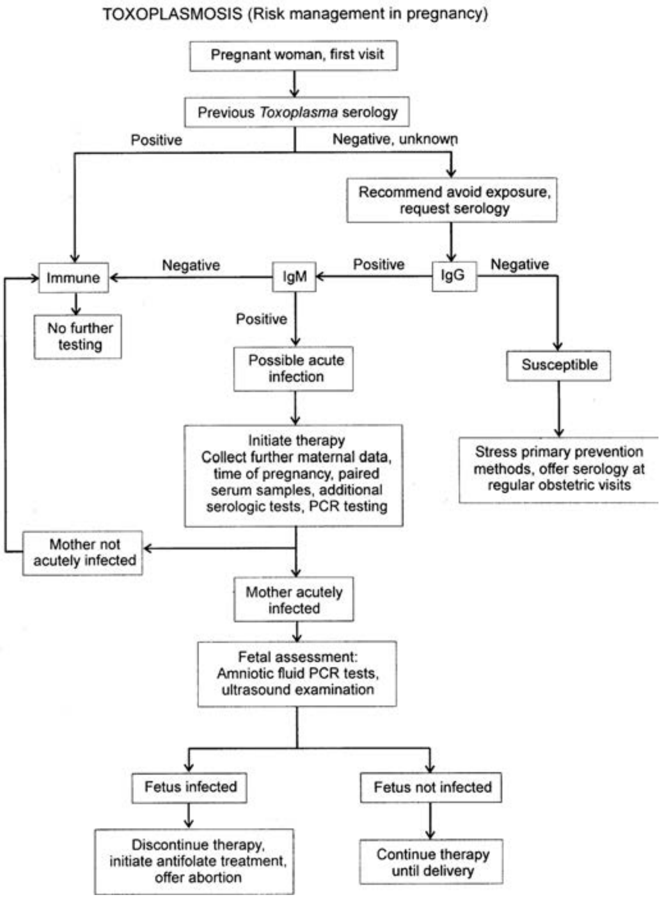

TOXOPLASMOSIS (Risk management in pregnancy)
Pregnant woman, first visit
Previous *Toxoplasma* serology
Positive
Negative, unknown
Recommend avoid exposure, request serology
Immune
Negative
IgM
Positive
IgG
Negative
No further testing
Positive
Possible acute infection
Susceptible
Initiate therapy Collect further maternal data, time of pregnancy, paired serum samples, additional serologic tests, PCR testing
Stress primary prevention methods, offer serology at regular obstetric visits
Mother not acutely infected
Mother acutely infected
Fetal assessment: Amniotic fluid PCR tests, ultrasound examination
Fetus infected
Fetus not infected
Discontinue therapy, initiate antifolate treatment, offer abortion
Continue therapy until delivery

Figure 25.17 Toxoplasmosis: risk management in pregnancy. doi:10.1128/9781555819002.ch25.f17

Treatment

It is generally recommended that the following conditions be treated: clinically active disease, diagnosed congenital toxoplasmosis, and disease in symptomatic compromised patients. Drugs that have been found to be effective, either alone or in combination, include sulfonamides and pyrimethamine (actively multiplying tachyzoites). Clindamycin and spiramycin have also been used in certain circumstances.

Pregnant Patients. Therapy for pregnant patients who acquire the infection and for newborns with *Toxoplasma* antibody is somewhat controversial. However, prophylactic therapy is often recommended for the newborn until it can be demonstrated that IgM antibody is not present. Spiramycin has been used to treat women infected in pregnancy, since it is potentially less toxic for the fetus. Apparently this drug is concentrated in the placenta but does not cross the barrier freely, and drug levels in the neonate are low. Spiramycin has also been used to treat congenitally infected infants every other month, alternating with sulfadiazine-pyrimethamine treatment.

Women who develop toxoplasmosis during the first trimester of pregnancy should be treated with spiramycin (3 to 4 g/day). After the first trimester, if there is no documented transmission to the fetus, spiramycin can be continued until term. Spiramycin is not currently available in the U.S. but can be obtained at no cost from Aventis through IND from the FDA (301-796-1600) following confirmation of the diagnosis by a recognized laboratory (i.e., Palo Alto Medical Foundation, Toxoplasmosis Laboratory; 650-853-4828) (24). If transmission has occurred *in utero*, therapy with pyrimethamine and sulfadiazine should be started (24). Pyrimethamine is a potential teratogen and should be used only after the first trimester (52). Congenitally infected newborns should be treated with pyrimethamine every 2 or 3 days and a sulfonamide daily for about 1 year (24, 53).

AIDS Patients. Although a combination of pyrimethamine and a sulfonamide is the standard regimen for treating TE in AIDS patients, the use of these drugs can be contraindicated in patients with a history of bone marrow suppression and severe allergic reactions to sulfonamides. It is also important to realize the potential danger of using high doses of sulfadiazine, which may lead to the development of sulfadiazine-induced crystalluria; to prevent crystalluria, patients should be adequately hydrated and their urinary pH should be maintained above 7.5. The use of oral clindamycin and pyrimethamine is effective (24). Data also suggest that a combination of trimethoprim and sulfamethoxazole (TMP-SMX, cotrimoxazole) may be as effective as pyrimethamine-sulfadiazine in AIDS patients with TE. It is interesting that treatment of cerebral toxoplasmosis protects against *Pneumocystis* pneumonia in AIDS patients; the decreased risk is probably due to chronic suppressive treatment with pyrimethamine and sulfonamides.

Approximately 10 to 50% of patients who are seropositive for antibodies to *T. gondii* have CD4$^+$ T-lymphocyte counts of <100/mm^3. Detection of IgG antibodies indicates prior infection and possible presence of tissue cysts. Since TE may result from reactivation of tissue cysts, prophylaxis with TMP-SMX or dapsone plus pyrimethamine with leucovorin is recommended for these patients. HIV-infected persons who are seronegative for *Toxoplasma* IgG should be counseled to protect themselves from primary infection by eating well-cooked meats and washing their hands after possible soil contact. Cats kept as pets should be fed commercial or well-cooked food, should be kept indoors, and should have their litter box changed each day. An algorithm for prevention of TE can be seen in Fig. 25.20. TE continues to occur among patients not receiving prophylaxis. It is estimated that at least half of the cases could be

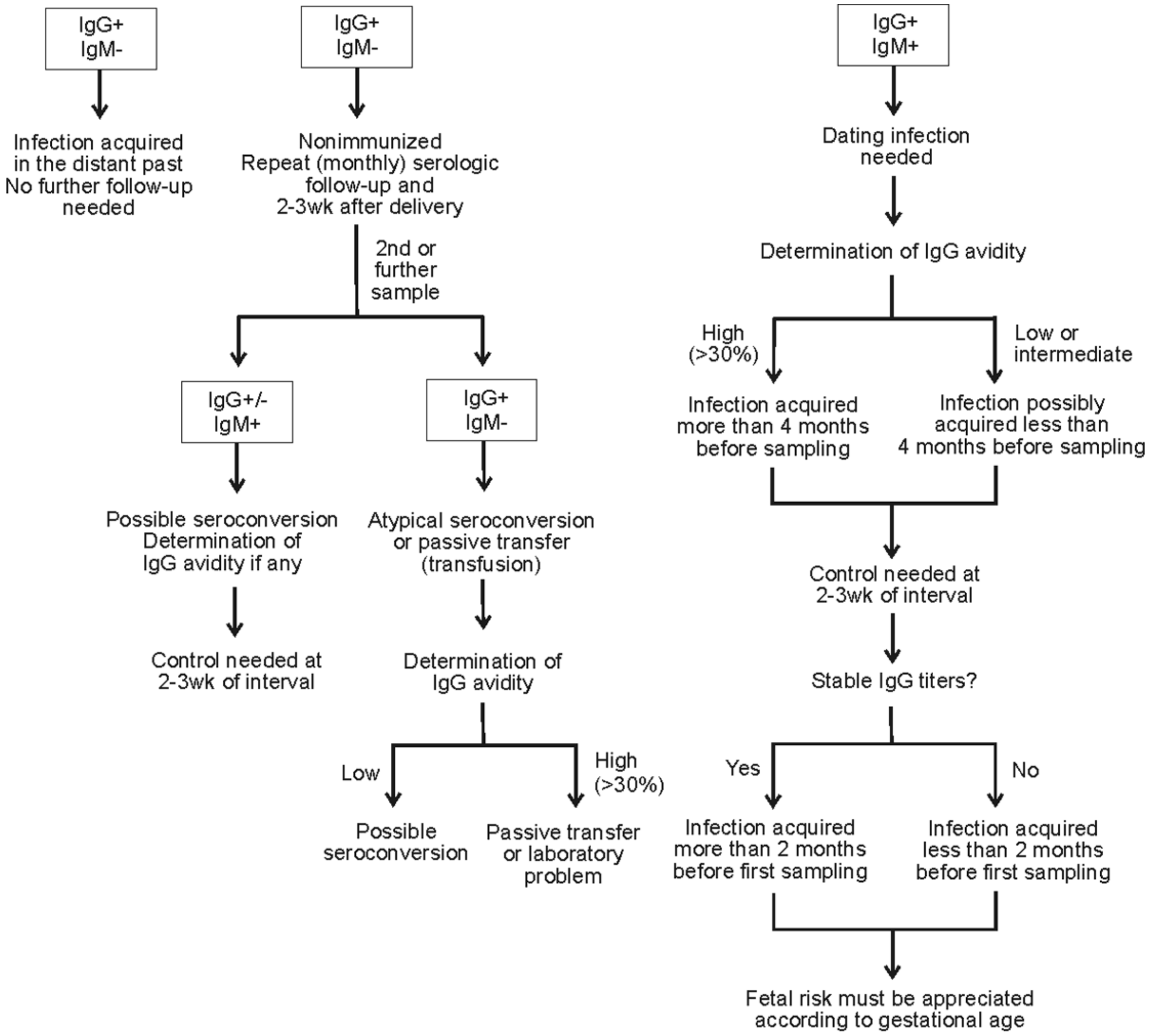

IgG+
IgM-
Infection acquired in the distant past No further follow-up needed
IgG+
IgM-
Nonimmunized Repeat (monthly) serologic follow-up and 2-3wk after delivery
2nd or further sample
IgG+/-
IgM+
Possible seroconversion Determination of IgG avidity if any
Control needed at 2-3wk of interval
IgG+
IgM-
Atypical seroconversion or passive transfer (transfusion)
Determination of IgG avidity
Low
High (>30%)
Possible seroconversion
Passive transfer or laboratory problem
IgG+
IgM+
Dating infection needed
Determination of IgG avidity
High (>30%)
Low or intermediate
Infection acquired more than 4 months before sampling
Infection possibly acquired less than 4 months before sampling
Control needed at 2-3wk of interval
Stable IgG titers?
Yes
No
Infection acquired more than 2 months before first sampling
Infection acquired less than 2 months before first sampling
Fetal risk must be appreciated according to gestational age

Figure 25.18 Chart for the analysis of conventional antibody tests for *Toxoplasma gondii* by the IgM technique. Interpretation depends on the type of test used. In the IgM-indirect immunofluorescence assay test, a titer of 1:64 would support a diagnosis of infection acquired 1 to 3 months earlier. In the double-sandwich IgM-ELISA (IgM capture), titers of 1:256 and higher should be expected in the first 4 months of infection. (Adapted from references 33, 39, and 41.) doi:10.1128/9781555819002.ch25.f18

prevented by better motivation of physicians and increased compliance by patients.

Ocular Toxoplasmosis. The management of ocular toxoplasmosis depends on the severity of the clinical presentation. Most specialists treat patients whose visual acuity has progressed to worse than 20/200, who have lesions in the peripapillary, perifoveal, or maculopapillary bundle regions, or who have severe vitreous inflammation. However, most do not treat patients whose visual acuity is 20/20, who have lesions in the far peripheral retina, or who have trace to mild vitreous inflammation. Various therapeutic regimens are used; however, almost all specialists, when asked, indicated that they used corticosteroids in the initial treatment regimen. In general, most indicated that they would modify treatment during pregnancy, in newborns, and in AIDS patients. Additional information can be found in chapter 36.

Epidemiology and Prevention

Human infection can be acquired through ingestion or handling of infected meat or through ingestion of infective oocysts, which can remain viable within cool, moist soil for a year or longer (Tables 25.5, 25.9). Certainly, hand

TABLE 25.8 Guide to interpretation of serologic tests for the diagnosis of toxoplasmosis[a]

	Infection status[c] of patients with titer[d] of:				
Clinical condition[b]	**0 (negative)**	**1:2–1:16**	**1:32–1:128**	**1:256–1:512**	**> 1:1,024**
Asymptomatic	Susceptible to infection	Infection in the past, probably immune	Infection in the past, probably immune	Infection in the past, probably immune	Recent infection or reinfection
Pregnancy	Susceptible to infection	Infection in the past, probably immune	Infection in the past, probably immune	Infection in the past, probably immune	Need to monitor neonate for infection
Newborn, asymptomatic or with jaundice	No	No	Diagnosis unlikely	Diagnosis unlikely	Possible, perform testing for IgM
Newborn with encephalitis	No	No	No	Diagnosis unlikely	Diagnosis may be correct if titer is stable or rises; perform testing for IgM
Lymphadenopathy	No	No	No	Diagnosis unlikely	Diagnosis possible
Fever with pneumonia, myocarditis, or hepatitis	No	No	Diagnosis unlikely	Diagnosis unlikely	Diagnosis possible; perform testing for IgM
Retinochoroiditis	No	Diagnosis possible	Diagnosis possible	Diagnosis possible	Diagnosis possible
Encephalitis	No	No	Diagnosis unlikely	Diagnosis unlikely	Possible; perform testing for IgM
Encephalitis (immunosuppressed patient)	Nondiagnostic	Diagnosis unlikely (may be tranfusion related)	Diagnosis possible	Diagnosis possible	Diagnosis likely; perform testing for IgM

[a] Adapted from reference 41.

[b] Patients are immunocompetent except where stated otherwise.

[c] 0, Susceptible to infection; the patient is not immune and is susceptible to a primary infection. 1:2–1:16, No; with these titers, toxoplasmosis should not be diagnosed. 1:32–1:128, Diagnosis unlikely; these titers would be the lowest found in typical forms of the syndrome. 1:256–1:512, Diagnosis possible; these titers would be characteristic but not necessarily diagnostic. >1:1,024, Diagnosis likely; these titers would probably be diagnostic for toxoplasmosis; perform testing for IgM as the presence of antibody may be informative.

[d] By dye test or indirect fluorescent-antibody test for IgG.

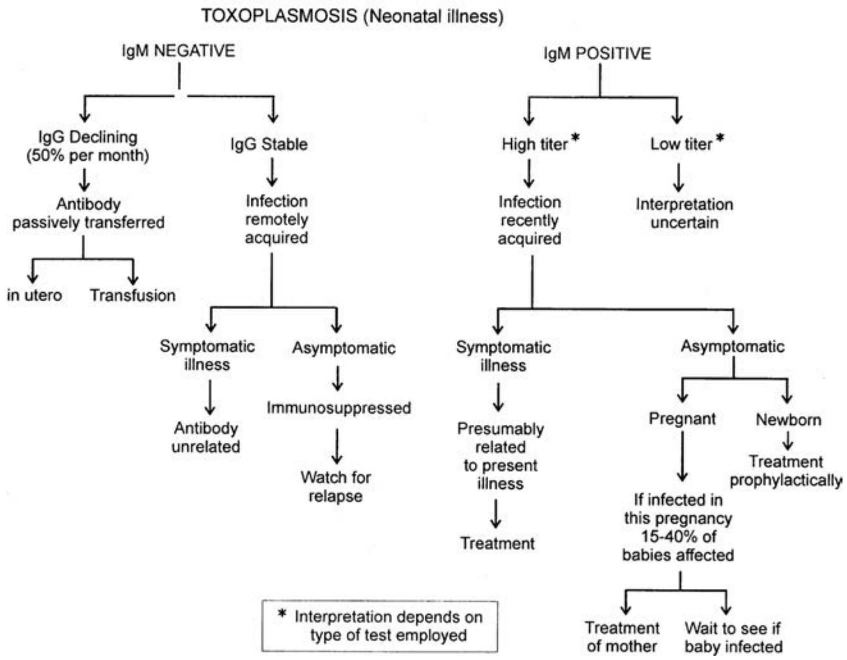
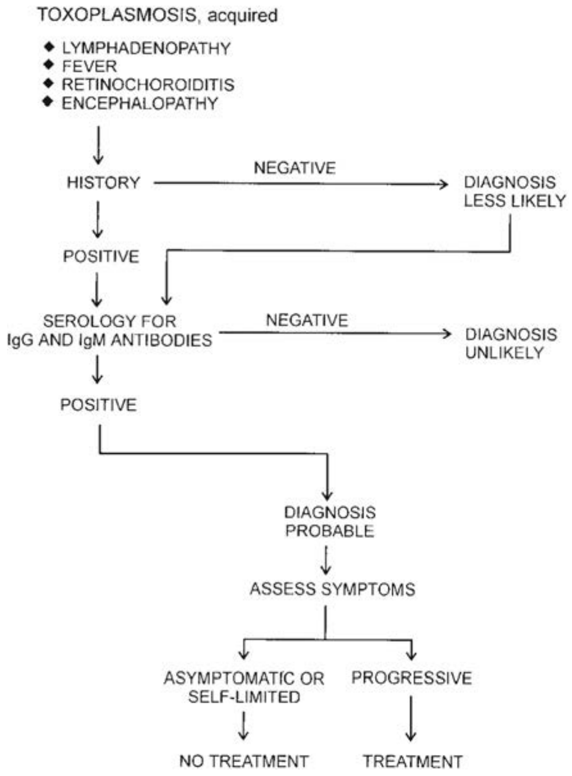

TOXOPLASMOSIS (Neonatal illness)
IgM NEGATIVE
IgM POSITIVE
IgG Declining (50% per month)
IgG Stable
High titer *
Low titer *
Antibody passively transferred
Infection remotely acquired
Infection recently acquired
Interpretation uncertain
in utero
Transfusion
Symptomatic illness
Asymptomatic
Symptomatic illness
Asymptomatic
Antibody unrelated
Immunosuppressed
Watch for relapse
Presumably related to present illness
Treatment
Pregnant
Newborn
Treatment prophylactically
If infected in this pregnancy 15-40% of babies affected
Treatment of mother
Wait to see if baby infected
* Interpretation depends on type of test employed

Figure 25.19 Interpretation of toxoplasmosis serology in pregnant women. (Courtesy of reference 35.) doi:10.1128/9781555819002.ch25.f19


TOXOPLASMOSIS, acquired
◆ LYMPHADENOPATHY
◆ FEVER
◆ RETINOCHOROIDITIS
◆ ENCEPHALOPATHY
HISTORY
NEGATIVE
DIAGNOSIS LESS LIKELY
POSITIVE
SEROLOGY FOR IgG AND IgM ANTIBODIES
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
DIAGNOSIS PROBABLE
ASSESS SYMPTOMS
ASYMPTOMATIC OR SELF-LIMITED
PROGRESSIVE
NO TREATMENT
TREATMENT

Algorithm 25.1 Toxoplasmosis.

Figure 25.20 Chart for the prevention of TE in HIV-positive patients; the approach is based on results of the *Toxoplasma* IgG serology. (Adapted from references 33, 39, and 41.) doi:10.1128/9781555819002.ch25.20

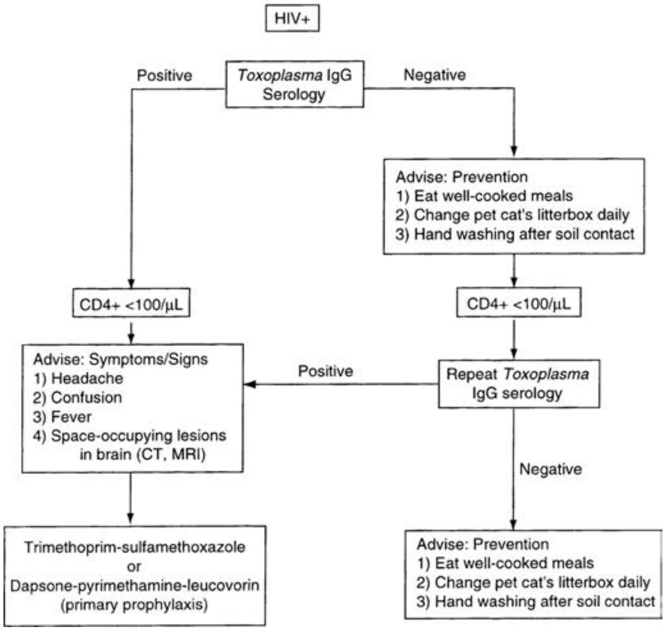

HIV+
Positive
Toxoplasma IgG Serology
Negative
Advise: Prevention
1) Eat well-cooked meals
2) Change pet cat's litterbox daily
3) Hand washing after soil contact
CD4+ <100/µL
CD4+ <100/µL
Advise: Symptoms/Signs
1) Headache
2) Confusion
3) Fever
4) Space-occupying lesions in brain (CT, MRI)
Positive
Repeat *Toxoplasma* IgG serology
Negative
Trimethoprim-sulfamethoxazole or Dapsone-pyrimethamine-leucovorin (primary prophylaxis)
Advise: Prevention
1) Eat well-cooked meals
2) Change pet cat's litterbox daily
3) Hand washing after soil contact

TABLE 25.9 Prevention of infection with *Toxoplasma gondii*

Prevention	Comments
Do not eat raw or undercooked meat or eggs	An example would be hollandaise sauce, prepared from raw eggs; pork, lamb, and venison particularly involved
Cured and smoked meat and sausages are considered safe	As an additional precaution, freeze at –20°C for a minimum of 1 day; however, some sausages are eaten almost raw after adding salt and spices (incidence of *T. gondii* in certain populations can be very high)
Consume only pasteurized or ultra-heat-treated milk and dairy products	Remember to consider ingredients of dairy products
When handling raw meat, avoid contaminating face, other food, utensils, cutting boards, or countertops	Hand washing and cleaning utensils, sinks, cutting boards, and countertops sufficient
Wash or peel fruits prior to eating them uncooked	Washing of smooth fruits is much easier than of berries; irradiation at 0.5 kGy is effective in "killing" coccidian oocysts on fruits and vegetables
Control insect pests and their access to food	Keep foods covered and/or refrigerated
Wear gloves when gardening or handling sand where cats usually defecate	Wash hands after removing gloves
If living with cats, change litter boxes every day and rinse them with boiling water	In one day, oocysts in cat feces will not have time to mature—extended periods in litter boxes will lead to fully infectious oocysts
Monitor and limit cat food sources	Infection in cats can be reduced by not allowing them to eat raw meat, birds, and rodents; use dry or canned cat food; in some areas of the world, ingestion of infected chicken viscera can lead to oocyst shedding and spread of *T. gondii* in the environment
Avoid handling stray cats and kittens	May be outdoor cats or may have consumed raw meat
Administer feline *T. gondii* vaccine to farm cats on swine operations	Reduction of exposure of finishing pigs to *T. gondii*; promising intervention to reduce risk of contaminating pork

washing is highly recommended when there has been potential exposure to the oocysts. It is recommended that meat be cooked so that the internal temperature reaches 150°F (66°C). The question of the house cat always arises; indoor cats fed on dry, canned, or boiled food are unlikely to be infected. However, cats that go outside and have access to other infected animals (birds or mice) may shed oocysts in their feces; therefore, other preventive measures, including changing the litter box daily and disinfecting the pan with boiling water, have been recommended. Also, the feces should not be placed onto the soil but, rather, should be disposed of either in the toilet or within bags.

Until recently, waterborne transmission of *T. gondii* was considered uncommon, but several large outbreaks linked to contaminated municipal water sources have led to a reassessment of this potential mode of transmission (33, 54). Considering the importance of using autofluorescence in environmental detection of oocysts, it has now been established that *T. gondii* oocysts fluoresce with the same pale blue color as can be seen with *Cyclospora cayetanensis* and other organisms. Apparently, morphologic differences among coccidian oocysts provide fluorescent details sufficient for identification when testing environmental water samples (55).

Since primary infection with *T. gondii* leads to specific and definitive protection against reinfection, the feasibility of developing a vaccine continues to be investigated. To protect humans, indirect procedures such as vaccination of the definitive host, i.e., the cat, or direct procedures by immunization with nonliving vaccines may be required.

Toxoplasma Types

Most *Toxoplasma* strains in North America and Europe fall into three clonal lineages known as types 1 (I), 2 (II), and 3 (III). South American strains are more genetically diverse and comprise distinct genotypes. The majority of human infections that have been studied in North America and Europe are caused by type II strains, which are also common in agricultural animals from these regions. However, several diverse genotypes of *T. gondii* are associated with severe infections in humans in South America.

In North America, type 2 strains are associated with congenital infections and AIDS patients. These same type 2 strains are also associated with human infections in Europe, whereas other diverse genotypes are found in other parts of the world.

Type 1 has been identified in patients in China, being the major type prevalent in mainland China, and has also been found in Columbia, Sri Lanka, Vietnam, and the U.S.; thus it is widespread in Asia and South and North America.

In a more recent study, PCR-RFLP genotype #1 (type II clonal), #2 (type III), #3 (type II variant), and #10 (type I) are found worldwide. Genotypes #2 and #3 dominate in Africa, genotypes #9 (Chinese 1) and #10 are prevalent in Asia, genotypes #1, #2, and #3 are prevalent in Europe, and genotypes #1, #2, #3, #4, and #5 dominate in North America (#4 and #5 are collectively known as type 12). In Central and South America, there is no clear dominance of any genotype even though a few have relatively higher frequencies. Statistically there are significant differences among populations in Africa, Asia, Europe, North America, and Central and South America, with only Europe and North America exhibiting similar diversity (56).

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26

Malaria and Babesiosis

Malaria

Babesiosis

Malaria

Malaria represents one of the oldest documented diseases of humans, and even today, organisms in the genus *Plasmodium* kill more people than do any other infectious microorganisms. Egyptian mummies more than 3,000 years old have been found with enlarged spleens, most probably due to malaria. Deadly periodic fevers and splenomegaly have been mentioned, dating from as early as 2700 BCE, in both Egyptian and Chinese writings.

Based on the lack of malaria records in the New World prior to the arrival of European explorers, conquistadors, and colonists, it is assumed that Europeans imported *Plasmodium malariae* and *Plasmodium vivax* to the Americas. Apparently, the arrival of *Plasmodium falciparum* coincided with the importation of African slaves, and by the early 1800s malaria was found worldwide.

Malaria has probably had a greater impact on world history than any other infectious disease. It has been responsible for the outcome of wars, population movements, and the growth and development of various nations throughout the world. Before the American Civil War, malaria was found as far north as southern Canada; however, there has been a gradual decline, and by the early 1950s it was no longer an endemic disease within the United States. It is still a very common disease in many parts of the world, particularly in tropical and subtropical areas (Table 26.1, Fig. 26.1).

Of the five species that infect humans, *P. vivax* and *P. falciparum* account for 95% of infections. Some estimates indicate that *P. vivax* may account for 80% of the infections. This species also has the widest distribution, extending throughout the tropics, subtropics, and temperate zones. *P. falciparum* is generally confined to the tropics, *P. malariae* is sporadically distributed, and *P. ovale* is confined mainly to central West Africa and some South Pacific islands.

We usually associate malaria with patients having a history of travel within an area where malaria is endemic. However, other situations that may result in infection involve the receipt of blood transfusions, use of hypodermic needles contaminated by prior use (as with, for example, drug addicts), possibly congenital infection, and transmission within the United States by indigenous mosquitoes that acquired the parasites from imported infections (1).

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TABLE 26.1 Countries reported by the Centers for Disease Control and Prevention to have areas of endemic malaria (2013)[a]

Afghanistan
Algeria
Angola
Argentina
Azerbaijan
Bangladesh
Belize
Benin
Bhutan
Bolivia
Botswana
Brazil
Burkina Faso
Burundi
Cambodia
Cameroon
Cape Verde
Central African Republic
Chad
China, People's Republic
Colombia
Comoros
Congo
Costa Rica
Cote d'Ivoire (formerly Ivory Coast)
Democratic People's Republic of Korea
Democratic Republic of the Congo
Djibouti
Dominican Republic
Ecuador
El Salvador
Equatorial Guinea
Eritrea
Ethiopia
French Guiana
Gabon
Gambia
Ghana
Guatemala
Guinea
Guinea-Bissau
Guyana
Haiti
Honduras
India
Indonesia
Iran (Islamic Republic of)
Iraq
Kenya
Kyrgyzstan
Lao People's Democratic Republic
Liberia
Madagascar
Malawi
Malaysia
Mali
Mauritania
Mayotte
Mexico
Mozambique
Myanmar (formerly Burma)
Namibia
Nepal
Nicaragua
Niger
Nigeria
Pakistan
Panama
Papua New Guinea
Paraguay
Peru
Philippines
Republic of Korea
Rwanda
São Tomé and Principe
Saudi Arabia
Senegal
Sierra Leone
Solomon Islands
Somalia
South Africa
South Sudan
Sri Lanka
Sudan
Suriname
Swaziland
Tajikistan
Thailand
Timor-Leste
Togo
Turkey
Uganda
United Republic of Tanzania (mainland)
United Republic of Tanzania (Zanzibar)
Uzbekistan
Vanuatu
Venezuela (Bolivarian Republic of)
Vietnam
Yemen
Zambia
Zimbabwe

[a] Courtesy of World Health Organization.

With the exception of tuberculosis, malaria kills more people than any other communicable disease in the world. More than 300 million to 500 million individuals throughout the world are infected with malaria, and 1.5 million to 2.7 million people a year, most of whom are children, are being killed by the disease. More people are dying from malaria today than 30 years ago. Malaria is endemic in over 100 countries in which 2,400 million people live; this is approximately 50% of the world's population (2). The African countries, where 90% of the malaria deaths occur, are ravaged by warfare, and the richer nations that fund most malaria research are cutting budgets. Malaria prevention is difficult and no drug is universally effective. Vaccine development studies are under way but malarial vaccines are not yet widely used.

Although over 1,000 cases of malaria are reported to the Centers for Disease Control and Prevention each year, the actual number of cases may be much larger. There has been a definite increase in the number of cases of *P. falciparum* malaria reported, which may be related to increased resistance to chloroquine. In 2011, 1,925 cases were reported in the United States, which represents an increase of 14% from 2010 and the largest number of reported cases since 1971 (1). Of these cases, 1,920 cases were classified as imported, 1 laboratory-acquired case, 1 transfusion-related case, 2 congenital cases, and 1 cryptic case. Over 49% of the cases were *P. falciparum*, with 22% *P. vivax*, 2.6% *P. malariae*, 2.6% *P. ovale*, and 1% mixed infections. During 2011, no cases of *P. knowlesi* were reported in the United States, although 23% of the cases overall were undetermined to the species level. There were 91 cases involving military personnel.

It is important for both physicians and laboratorians within areas where malaria is not endemic to be aware of the difficulties related to malaria diagnosis and the fact that symptoms are often nonspecific and may mimic other

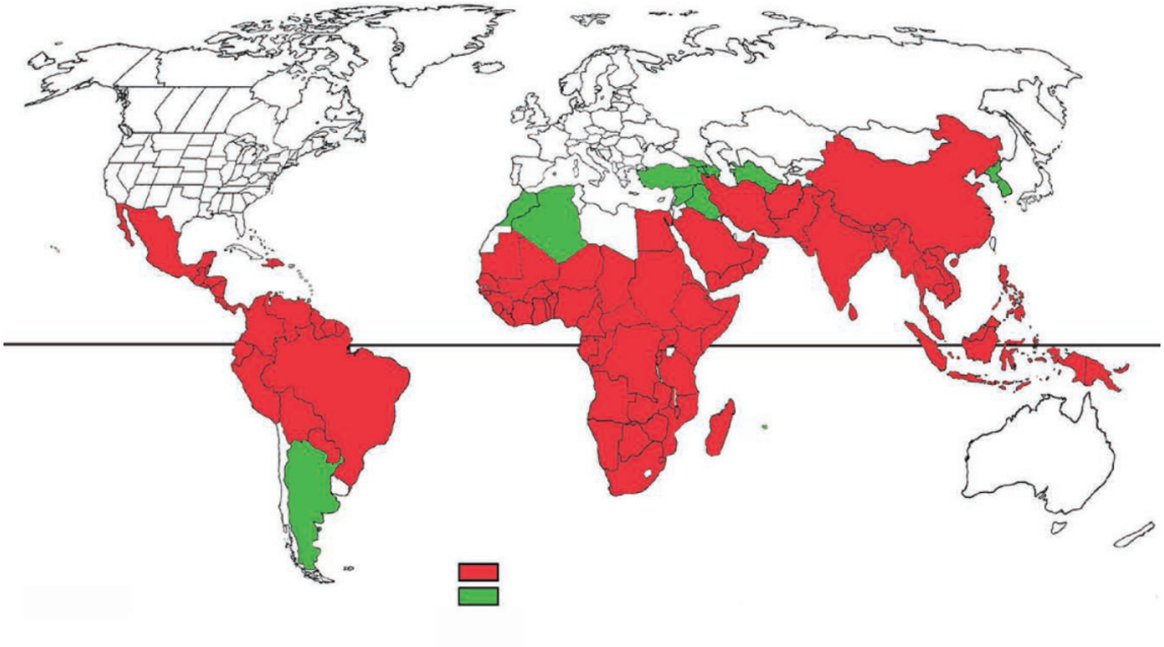


Figure 26.1 Countries where malaria is endemic; green indicates regions where *Plasmodium vivax* only is endemic (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch26.f1

problems. It is important for physicians to recognize that travelers are susceptible to malarial infection when they go to a country where malaria is endemic, and they should receive prophylactic medication. It is also important to remember that 80% of the *P. falciparum* cases acquired by American civilians are contracted in sub-Saharan Africa; currently there are 40 cities in Africa with over 1 million inhabitants, and by 2025 over 800 million people will live in urban areas. Malaria has always been a rural disease in Africa; however, it appears that the urban poor are at a much higher risk of contracting malaria than was previously recognized. These changes also have the potential to affect travelers to Africa.

With the tremendous increase in the number of people traveling from the tropics to malaria-free areas, the number of imported malaria cases is also on the rise. There have been reports of imported infected mosquitoes transmitting the infection among people who live or work near international airports. It is also possible that mosquitoes can reach areas far removed from the airports (3). This situation has been termed "airport malaria," i.e., malaria that is acquired through the bite of an infected anopheline mosquito by persons with apparently no risk factors for the disease. Unfortunately, unless a careful history is obtained, the diagnosis of malaria can be missed or delayed. Tests to exclude malaria should be considered for patients who work or live near an international airport and who present with an acute febrile illness. The potential danger of disseminating the mosquito vectors of malaria via aircraft is well recognized; however, modern disinfection procedures have not yet eliminated the risk of vector transportation. Not only can insects survive nonpressurized air travel, but also they may be transported further by car or other means after arriving at the airport. Contraction of airport malaria is certainly an unusual event, particularly considering that the number of air travelers is well over 1 billion annually. However, in addition to asking a patient, "Where have you been, and when were you there?" one should ask, "Where do you live?"

Potential contributing factors to malaria-related fatalities in U.S. travelers include (i) failure to seek pretravel advice, (ii) failure to prescribe correct chemoprophylaxis, (iii) failure to obtain prescribed chemoprophylaxis, (iv) failure to adhere to the chemoprophylaxis regimen, (v) failure to seek medical care promptly for their illness, (vi) failure to obtain an adequate patient history, (vii) delay in diagnosis of malaria, and (viii) delay in initiating treatment of malaria (4). One or more of these potential problems can lead to the death of the patient.

Life Cycle and Morphology

Pre-Erythrocytic Cycle. The vector for malaria is the female anopheline mosquito. When the vector takes a blood meal, sporozoites contained in the salivary glands of the

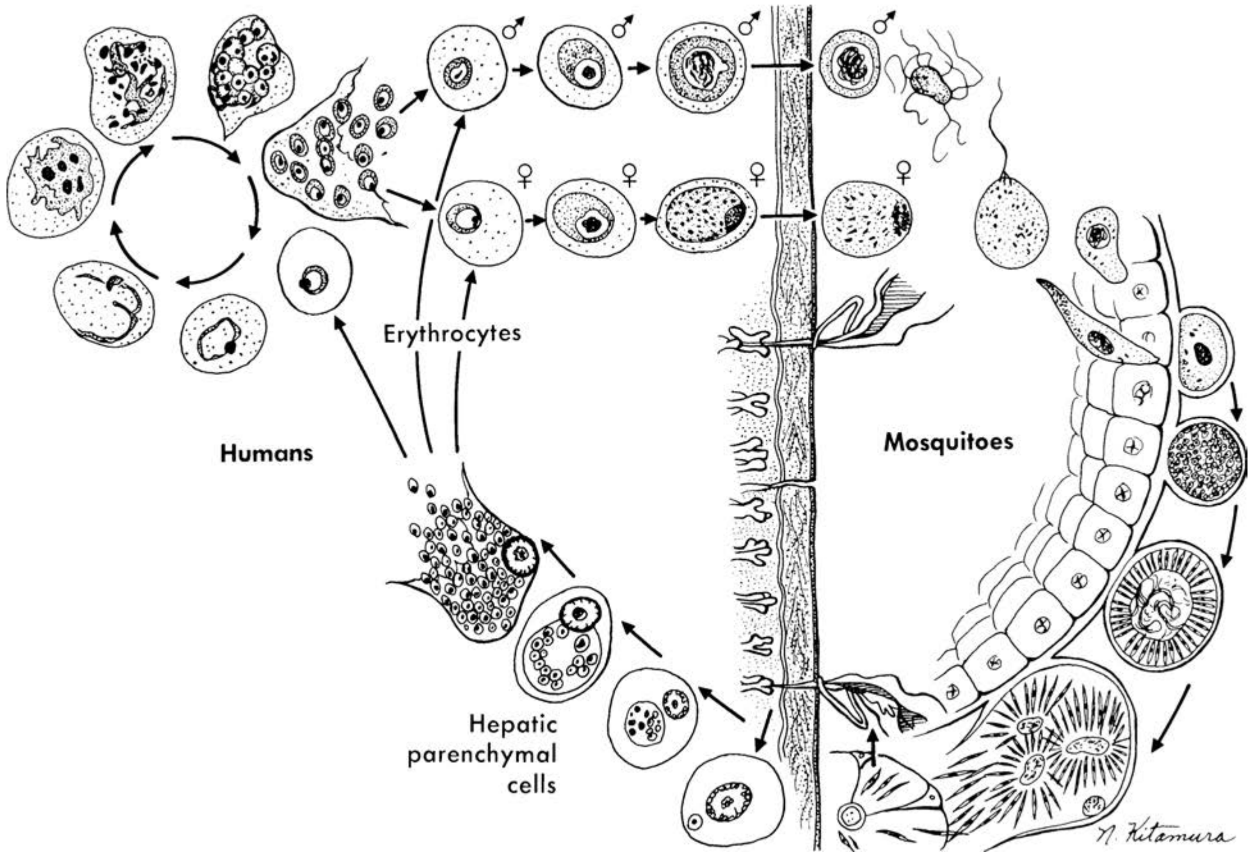
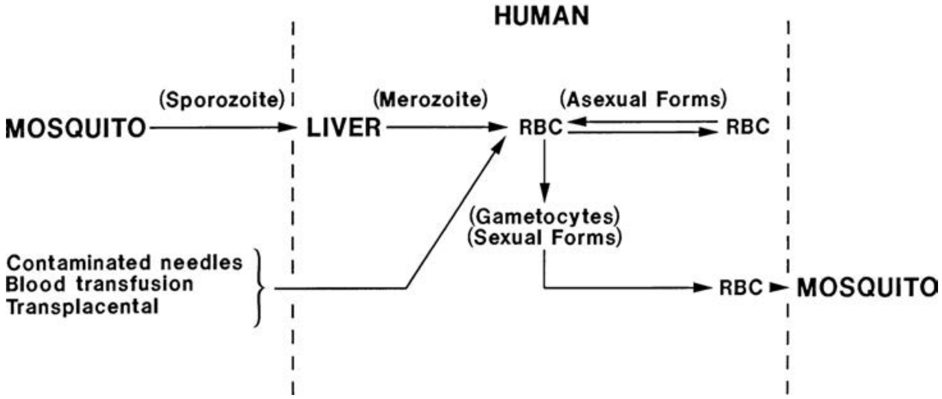

♂
♂
♂
♂
♀
♀
♀
♀
Erythrocytes
Humans
Mosquitoes
Hepatic parenchymal cells
N. Kitamura

Figure 26.2 Life cycle of *Plasmodium* spp. (Illustration by Nobuko Kitamura; adapted from Wilcox A, *Manual for the Microscopical Diagnosis of Malaria in Man*, U.S. Public Health Service bulletin 180, National Institutes of Health, Bethesda, MD, 1951.) doi:10.1128/9781555819002.ch26.f2

mosquito are discharged into the puncture wound (Fig. 26.2 and 26.3). Within an hour, these infective stages are carried via the blood to the liver, where they penetrate hepatocytes and begin to grow, thus initiating the pre-erythrocytic or primary exoerythrocytic cycle. Detailed study of sporozoite entry into the hepatocytes indicates that the process involves parasite-encoded surface proteins and host molecules. The sporozoites become round or oval and begin dividing repeatedly. This schizogony results in large numbers of exoerythrocytic merozoites.

Erythrocytic Cycle. Once these merozoites leave the liver, they invade the red blood cells (RBCs), thus initiating the erythrocytic cycle (Fig. 26.4 and 26.5). A secondary or

Figure 26.3 Life cycle of malaria. doi:10.1128/9781555819002.ch26.f3


HUMAN
(Sporozoite)
MOSQUITO
LIVER
(Merozoite)
RBC
(Asexual Forms)
RBC
(Gametocytes)
(Sexual Forms)
Contaminated needles
Blood transfusion
Transplacental
RBC
MOSQUITO

dormant schizogony may occur in *P. vivax* and *P. ovale* organisms, which remain quiescent in the liver until a later time. These resting stages have been termed hypnozoites (5). Delayed schizogony does not occur in *P. falciparum* and probably does not occur in *P. malariae* or *P. knowlesi*.

Recrudescence. The situation in which the RBC infection is not eliminated by the immune system or by therapy and the number in the RBCs begins to increase again with subsequent clinical symptoms is called a recrudescence. All species may cause a recrudescence.

True relapse. The situation in which the erythrocytic infection is eliminated and a relapse occurs later because of a new invasion of the RBCs from liver merozoites, called a recurrence or true relapse, theoretically occurs only in *P. vivax* and *P. ovale* infections.

Once the RBCs and reticulocytes have been invaded, the parasites grow and feed on hemoglobin. Within the RBC, the merozoite (or young trophozoite) is vacuolated, ring shaped, more or less ameboid, and uninucleate. The excess protein, an iron porphyrin, and hematin left over from the metabolism of hemoglobin combine to form malarial pigment (some workers use the term "hemozoin"; however, the term "malarial pigment" is used throughout this chapter).

Schizogony. Once the nucleus begins to divide, the trophozoite is called a developing schizont. The mature schizont contains merozoites (whose number depends on the species), which are released into the bloodstream. Many of the merozoites are destroyed by the immune system, but others invade RBCs, in which a new cycle of erythrocytic schizogony begins.

After several erythrocytic generations, some of the merozoites do not become schizonts but, rather, begin to undergo development into the male and female gametocytes. Whether this development is predetermined genetically or occurs as a response to some specific stimulus is unknown (6). It is likely that cells are committed to sexual development in the preceding round of asexual schizogony within the pre-erythrocytic schizont rather than differentiating following invasion of an RBC by uncommitted merozoites. Data also suggest that genetic and environmental factors may predispose *Plasmodium* spp. to switch from asexual to sexual development. Apparently, sexual commitment is highest in merozoites derived directly from the liver schizont, and there is a progressive loss of commitment with increasing numbers of asexual cycles. Environmental stimuli may relate directly to an increase in parasite density; there may also be some direct links between sublethal doses of chloroquine and an increase in gametocytogenesis, particularly in chloroquine-resistant parasites.

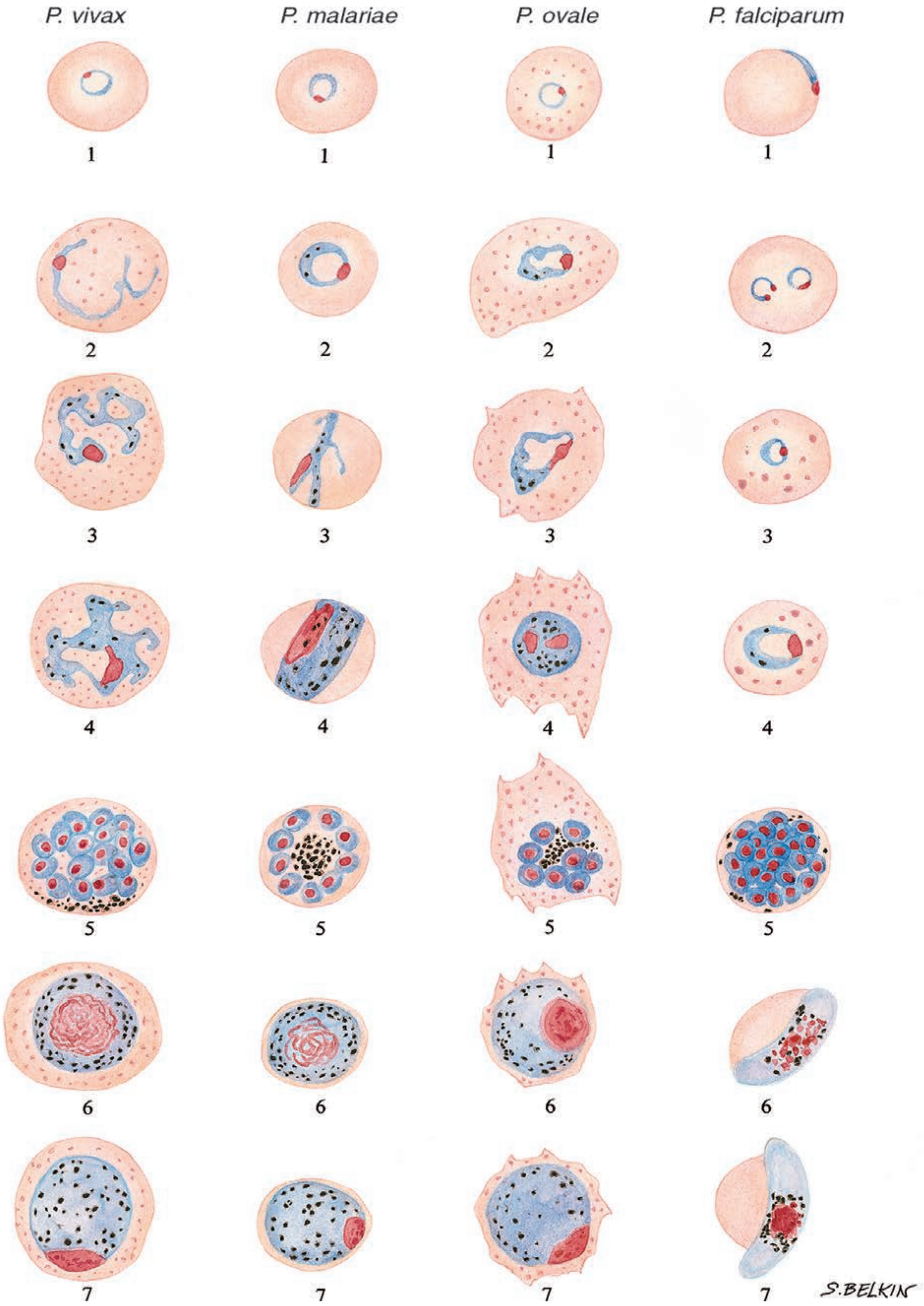
The asexual and sexual forms just described circulate in the bloodstream during infections by four of the *Plasmodium* species. However, in *P. falciparum* infections, as the parasite continues to grow, the RBC membrane becomes sticky and the cells tend to adhere to the endothelial lining of the capillaries of the internal organs. Thus, only the ring forms and the gametocytes (occasionally mature schizonts) normally appear in the peripheral blood.

Sporogony. If gametocytes are ingested when the mosquito takes a blood meal, they mature into gametes while in the mosquito gut. The male microgametes undergo nuclear division by a process called exflagellation, in which the microgametes break out of the RBC, become motile, and penetrate the female macrogamete, with the fertilized stage being called the zygote (Fig. 26.6). This zygote then becomes elongate and motile and is called the ookinete. This stage migrates to the mosquito midgut, secretes a thin wall, and grows into the oocyst, which extends into the insect's hemocele. Within a few days to 2 weeks, the oocyst matures, with the formation of hundreds to thousands of sporozoites. When the oocyst ruptures, the sporozoites are released into the hemocele and dispersed throughout the body, and some make their way into the salivary glands. When the mosquito next takes a blood meal, the sporozoites are injected with saliva into the host (Fig. 26.7).

Life Cycle within a Tube of Blood (EDTA). Unfortunately, when blood tubes are held at room temperature, especially with the cap removed, the process of exflagellation can occur within the blood. Subsequently, when the blood films are prepared, these microgametocytes can be confused with *Borrelia* spp. (7, 8). While the organisms are within the tube of blood, the life cycle continues, unlike the situation when fingerstick blood films are prepared. Thus, any delay in thick and thin blood film preparation can result in a loss of organisms, as well as multiple artifact problems.

Clinical Disease

From the time of the original mosquito bite until a week or more later, the patient remains asymptomatic. During this time, the organisms are undergoing multiplication (pre-erythrocytic cycle) in the liver. When the liver merozoites invade the RBCs, several broods begin to develop; however, one will eventually dominate and suppress the others, thus beginning the process of periodicity; this periodicity is very rarely seen in an immunologically naive patient early in the infection. Therefore, these patients with no prior exposure to malaria tend to be symptomatic early with a very low parasitemia and most often do not present as a possible malaria patient. Once the cycle is synchro-


P. vivax
P. malariae
P. ovale
P. falciparum
1
1
1
1
2
2
2
2
3
3
3
3
4
4
4
4
5
5
5
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S.BELKIN

nized, the simultaneous rupture of a large number of RBCs and liberation of metabolic waste by-products into the bloodstream precipitate the paroxysms of malaria.

Clinical symptoms of malaria include anemia, splenomegaly, and the classic paroxysm, with its cold stage, fever, and sweats. Although the febrile paroxysms strongly suggest infection, many patients who are seen in medical facilities, particularly in the early stages of the infection, do not exhibit the typical fever pattern. They may have fever or several small, random peaks each day. Since the symptoms associated with malaria are so nonspecific, the diagnosis should be considered in any symptomatic patient with a history of travel to an area where malaria is endemic. During the primary infection in a nonimmune host, the early fever episodes can affect density-dependent regulation of the parasite population, maintaining cycles of parasitemia and promoting synchronous parasite growth. The typical paroxysm begins with the cold stage and rigors lasting 1 to 2 h. During the next few hours, the patient spikes a high fever and feels very hot, and the skin is warm and dry. The last several hours are characterized by marked sweating and a subsequent drop in body temperature to normal or subnormal.

Anemia seen in malarial infections can be caused by a number of mechanisms, including (i) direct RBC lysis as a function of the life cycle of the parasite, (ii) splenic removal of both infected and uninfected RBCs (coated with immune complexes), (iii) autoimmune lysis of coated infected and uninfected RBCs, (iv) decreased incorporation of iron into heme, (v) increased fragility of RBCs, and (vi) decreased RBC production from bone marrow suppression.

Malaria can mimic many other diseases, such as gastroenteritis, pneumonia, meningitis, encephalitis, or hepatitis. Other possible symptoms include lethargy, anorexia, nausea, vomiting, diarrhea, and headache. Leukopenia can also be seen in malaria, as can an occasional elevated white blood cell (WBC) count with a left shift. Eosinophilia and thrombocytopenia may also be seen but are much less frequent. A clinical comparison of some of the features of the five different malarias is presented in Table 26.2. Information on the pathogenesis of malaria is provided in Fig. 26.8 and Tables 26.3 to 26.5.

***Plasmodium vivax*.** The primary clinical attack usually occurs 7 to 10 days after infection, although there are strain differences, with a much longer incubation period being possible. In some patients, symptoms such as headache, photophobia, muscle aches, anorexia, nausea, and sometimes vomiting occur before organisms can be detected in the bloodstream. In other patients, the parasites can be found in the bloodstream several days before symptoms appear (Tables 26.6 and 26.7).

During the first few days, the patient may not exhibit a typical paroxysm pattern but, rather, may have a steady low-grade fever or an irregular remittent fever pattern. Once the typical paroxysms begin, after an irregular periodicity, a regular 48-h cycle is established. An untreated primary attack may last from 3 weeks to 2 months or longer. The paroxysms become less severe and more irregular in frequency and then stop altogether. In 50% of patients, relapses occur after weeks, months, or up to 5 years (or more).

Severe complications are rare in *P. vivax* infections, although coma and sudden death or other symptoms of cerebral involvement have been reported. These patients can exhibit cerebral malaria, renal failure, circulatory collapse, severe anemia, hemoglobinuria, abnormal bleeding, acute respiratory distress syndrome, and jaundice (8, 9). Studies have confirmed that these were not mixed infections with *P. falciparum* but single-species infections with *P. vivax*. Recent data demonstrate that the infection comes

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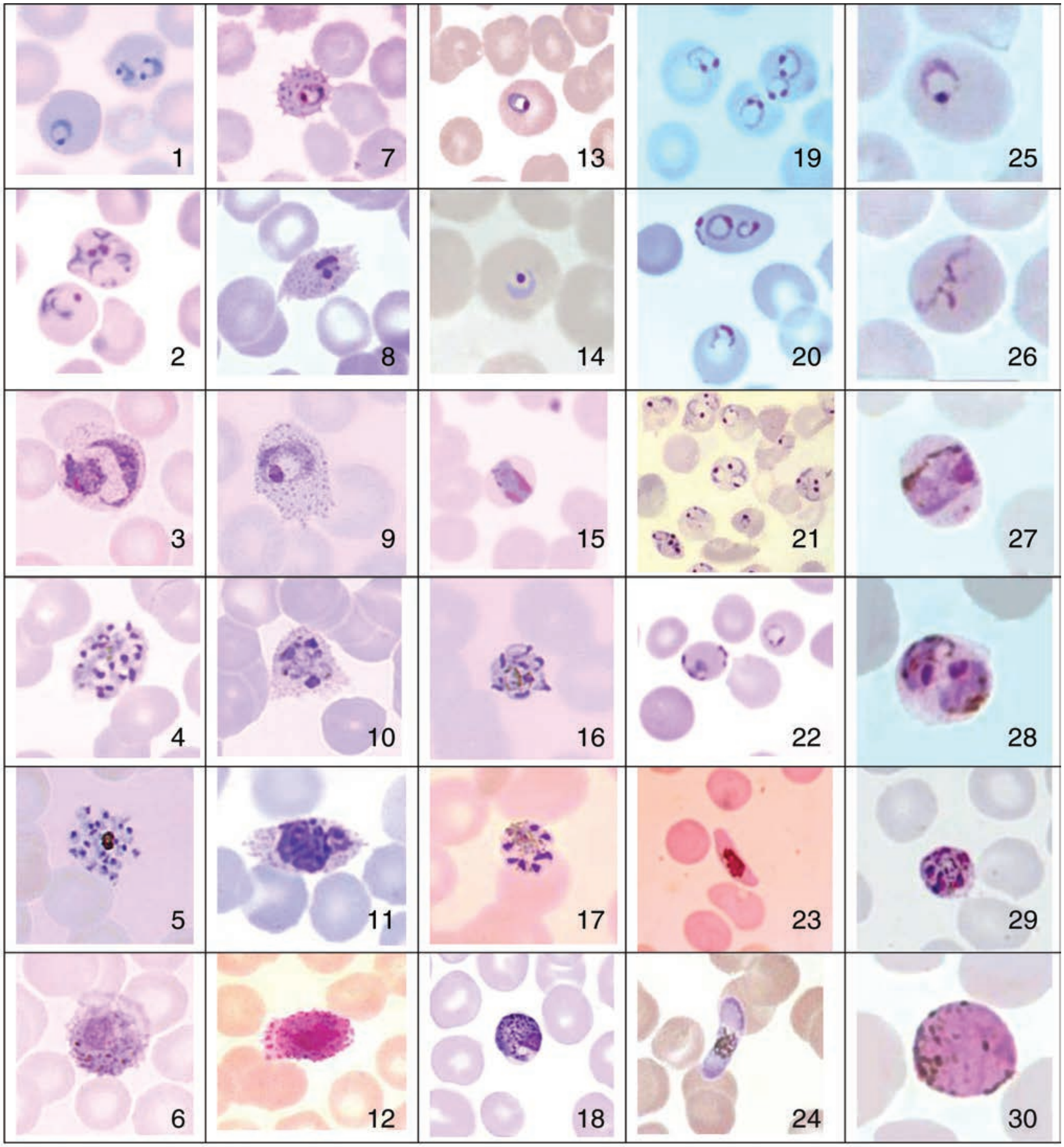
Figure 26.4 Morphology of malaria parasites. *Plasmodium vivax* (reading down): (1) early trophozoite (ring form); (2) late trophozoite with Schüffner's dots (note enlarged RBC); (3) late trophozoite with ameboid cytoplasm (very typical of *P. vivax*); (4) late trophozoite with ameboid cytoplasm; (5) mature schizont with 18 merozoites and clumped pigment; (6) microgametocyte with dispersed chromatin; (7) macrogametocyte with compact chromatin. *Plasmodium malariae*: (1) early trophozoite (ring form); (2) early trophozoite with thick cytoplasm; (3) early trophozoite (band form); (4) late trophozoite (band form) with heavy pigment; (5) mature schizont with nine merozoites arranged in a rosette; (6) microgametocyte with dispersed chromatin; (7) macrogametocyte with compact chromatin. *Plasmodium ovale*: (1) early trophozoite (ring form) with Schüffner's dots; (2) early trophozoite (note enlarged RBC); (3) late trophozoite in RBC with fimbriated edges; (4) developing schizont with irregularly shaped RBC; (5) mature schizont with eight merozoites arranged irregularly; (6) microgametocyte with dispersed chromatin; (7) macrogametocyte with compact chromatin. *Plasmodium falciparum*: (1) early trophozoite (accolé or appliqué form); (2) early trophozoite (one ring is in the headphone configuration with double chromatin dots); (3) early trophozoite with Maurer's dots; (4) late trophozoite with larger ring and Maurer's dots; (5) mature schizont with 24 merozoites; (6) microgametocyte with dispersed chromatin; (7) macrogametocyte with compact chromatin. **Note:** Without the appliqué form, Schüffner's dots, multiple rings per cell, and other developing stages, differentiation among the species can be very difficult. It is obvious that the early rings of the four species shown in this figure can mimic one another very easily. *Remember: one set of negative blood films cannot rule out a malaria infection.* doi:10.1128/9781555819002.ch26.f4

with a significant burden of morbidity and associated mortality.

Since *P. vivax* infects only the reticulocytes, the parasitemia is usually limited to around 2 to 4% of the available RBCs (Fig. 26.9). Splenomegaly occurs during the first few weeks of infection, and the spleen progresses from being soft and palpable to hard, with continued enlargement during a chronic infection. If the infection is treated during the early phases, the spleen returns to its normal size.

Leukopenia is usually present; however, leukocytosis may be present during the febrile episodes. Concentrations of total plasma proteins are unchanged, although the albumin level may be low and the globulin fraction may be elevated. The increase in the concentration of gamma glob-



ulins is caused by the development of antibodies. The level of potassium in serum may also be increased as a result of RBC lysis.

***Plasmodium ovale*.** Molecular methods have confirmed the existence of two distinct nonrecombining species of *Plasmodium ovale* (classic type *Plasmodium ovale curtisi* and variant type *Plasmodium ovale wallikeri*). In one study, a significant finding was more severe thrombocytopenia among patients with *P. ovale wallikeri* infection than among those with *P. ovale curtisi* infection. Recent epidemiologic studies conducted by using PCR techniques have found *P. ovale* infections in most of sub-Saharan Africa, Southeast Asia, and the Indian subcontinent, including prevalence as high as 15% according to results of studies conducted in rural Nigeria and Papua New Guinea. In addition, severe complications such as spleen rupture, severe anemia, or acute respiratory distress syndrome may occur in patients with *P. ovale* malaria. Thus, the global burden of *P. ovale* infection might have been underestimated.

Although *P. ovale* and *P. vivax* infections are clinically similar, *P. ovale* malaria is usually less severe, tends to relapse less frequently, and usually ends with spontaneous recovery, often after no more than 6 to 10 paroxysms (Tables 26.6 and 26.7). Relapses occur as early as 17 days after treatment of the primary attack to as late as 255 days. Delayed primary attacks occur when the primary attack has been eliminated, usually with antimalarial drugs. Such infections have been reported after 4 years (Fig. 26.10).

The incubation period is similar to that seen in *P. vivax* malaria, but the frequency and severity of the symptoms are much lower, with a lower fever and a lack of typical rigors. *P. ovale* infects only the reticulocytes (as does *P. vivax*), so that the parasitemia is generally limited to around 2 to 4% of the available RBCs (10).

The geographic range has been thought to be limited to tropical Africa, the Middle East, Papua New Guinea, and Irian Jaya in Indonesia. However, infections with *P. ovale* in Southeast Asia may be the cause of benign and relapsing malaria in this region. In both Southeast Asia and Africa, two different types of *P. ovale* circulate in humans. Human infections with variant-type *P. ovale* are associated with higher parasitemias and thus have possible clinical relevance (11).

The Duffy blood group does not appear to be a controlling factor for infections with *P. ovale* as it does with *P. vivax*. There appears to be no difference in susceptibility to infection between Caucasians and African-Americans. Because of the resistance of individuals with negative Duffy blood group to infection with *P. vivax* and the high prevalence of negativity in populations of West Africa, surveys reporting *P. vivax* may actually represent infections with *P. ovale*.

***Plasmodium malariae*.** *Plasmodium malariae* invades primarily the older, more mature RBCs, so that the number of infected cells is somewhat limited (Fig. 26.11). The incubation period between infection and symptoms may be much longer than that seen with *P. vivax* or *P. ovale* malaria, ranging from about 27 to 40 days. Parasites can be found in the bloodstream several days before the initial attack, and the prodromal symptoms may resemble those of *P. vivax* malaria. A regular periodicity is seen from the beginning, with a more severe paroxysm, including a

Figure 26.5 Morphology of malaria parasites. Morphology of malaria parasites. Column 1 (top to bottom), *Plasmodium vivax* (note enlarged infected RBCs): (1) early trophozoite (ring form) (note one RBC contains two rings—not that uncommon); (2) older ring, note ameboid nature of rings; (3) late trophozoite with Schüffner's dots (note enlarged RBC); (4) developing schizont; (5) mature schizont with 18 merozoites and clumped pigment; (6) microgametocyte with dispersed chromatin. Column 2 (top to bottom), *Plasmodium ovale* (note enlarged infected RBCs): (7) early trophozoite (ring form) with Schüffner's dots (RBC has fimbriated edges); (8) early trophozoite (note enlarged RBC, Schüffner's dots, and RBC oval in shape); (9) late trophozoite in RBC with fimbriated edges; (10) developing schizont with irregular-shaped RBC; (11) mature schizont with eight merozoites arranged irregularly; (12) microgametocyte with dispersed chromatin. Column 3, *Plasmodium malariae* (note normal or smaller than normal infected RBCs): (13) early trophozoite (ring form); (14) early trophozoite with thick cytoplasm; (15) late trophozoite (band form); (16) developing schizont; (17) mature schizont with nine merozoites arranged in a rosette; (18) macrogametocyte with compact chromatin. Column 4, *Plasmodium falciparum*: (19) early trophozoites (the rings are in the headphone configuration with double chromatin dots); (20) early trophozoite (accolé or appliqué form); (21) early trophozoites (note the multiple rings per cell); (22) late trophozoite with larger ring (accolé or appliqué form); (23) crescent-shaped gametocyte; (24) crescent-shaped gametocyte. Column 5, *Plasmodium knowlesi*—with the exception of image 29, these were photographed at a higher magnification (note normal or smaller than normal infected RBCs) (courtesy of the CDC Public Health Image Library): (25) early trophozoite (ring form); (26) early trophozoite with slim band form; (27) late trophozoite (band form); (28) developing schizont; (29) mature schizont with merozoites arranged in a rosette; (30) microgametocyte with dispersed chromatin. **Note:** Without the appliqué form, Schüffner's dots, multiple rings per cell, and other developing stages, differentiation among the species can be very difficult. It is obvious that the early rings of all four species can mimic one another very easily. Remember: one set of negative blood films cannot rule out a malaria infection. (Figure courtesy of Garcia LS, *Clin Lab Med* 30:93–128, 2010.) doi:10.1128/9781555819002.ch26.f5

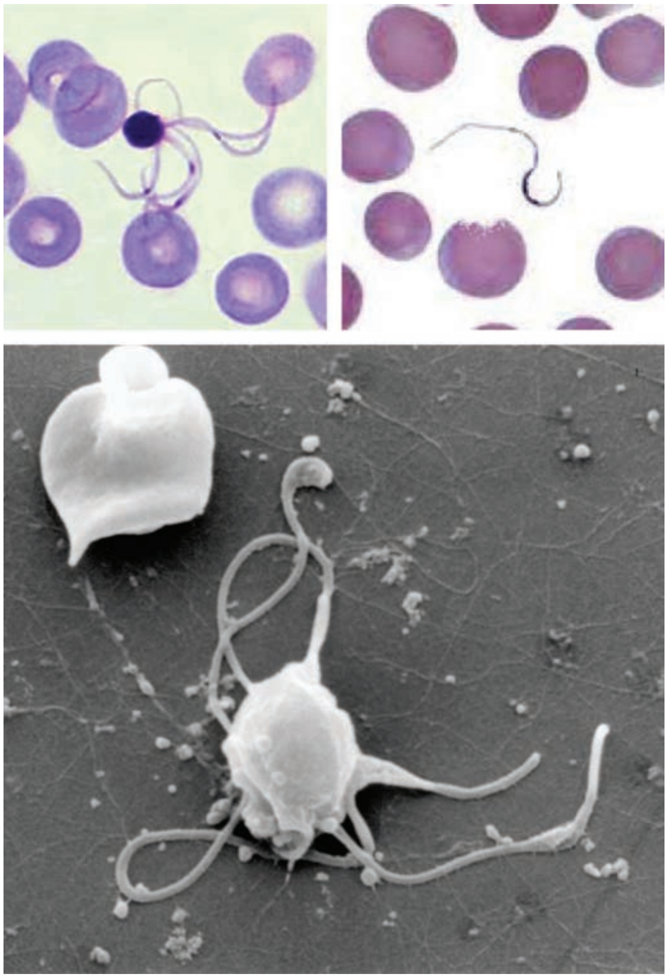


Figure 26.6 Exflagellating male gametocytes. (Upper, left) Giemsa stain; (right) single organism that can mimic a treponeme. (Lower) Scanning electron micrograph of exflagellating male gametocyte (courtesy of Robert E. Sinden).
doi:10.1128/9781555819002.ch26.f6

longer cold stage and more severe symptoms during the hot stage. Collapse during the sweating phase is not uncommon (Tables 26.6 and 26.7).

Proteinuria is common in patients with *P. malariae* infection; in children it may be associated with clinical signs of the nephrotic syndrome. It has been suggested that kidney problems may result from deposition within the glomeruli of circulating antigen-antibody complexes in an antigen excess situation seen with a chronic infection. Apparently, the nephrotic syndrome associated with *P. malariae* infections is unaffected by the administration of steroids. A membranoproliferative type of glomerulonephritis with relatively sparse proliferation of endothelial and mesangial cells is the most common type of lesion seen in *P. malariae* infection. Granular deposits of IgM, IgG, and C3 are seen in immunofluorescence assays (12, 13). Since chronic glomerular disease associated with *P. malariae* infections is usually not reversible with therapy, genetic and environmental factors may play a role in the nephrotic syndrome.

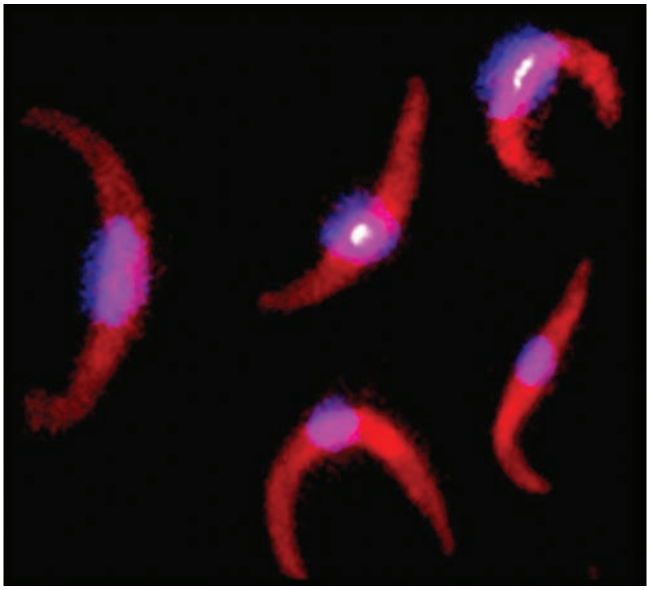


Figure 26.7 Malarial sporozoites from the mosquito vector.
doi:10.1128/9781555819002.ch26.f7

The infection may end with spontaneous recovery, or there may be a recrudescence or series of recrudescences over many years. These patients are left with a latent infection and persisting low-grade parasitemia for many, many years. *P. malariae* does not relapse from persistent liver stage parasites. However, the blood stage parasites persist for extremely long periods, perhaps for the life of the human host. There have been many reports of people who have left zones of endemicity whose infections have recrudesced, under stress, after many years of dormancy, or who have donated blood and the recipient has developed an infection. It is documented that these cases can recrudesce as long as 50 to 70 years after the original infection. Because almost all of these long-term infections have been detected following transfusion donations, it is believed that the parasites have persisted in the blood at very low densities with the patients remaining asymptomatic the entire time.

Plasmodium falciparum**.** *P. falciparum* tends to invade all ages of RBCs, and the proportion of infected cells may exceed 50%. Schizogony occurs in the internal organs (spleen, liver, bone marrow, etc.) rather than in the circulating blood. Ischemia caused by the plugging of vessels within these organs by masses of parasitized RBCs produces various symptoms, depending on the organ involved (Fig. 26.12). It has been suggested that a decrease in the

TABLE 26.2 *Plasmodium* spp.: characteristics of the five infections[a]

Infection parameter	*P. vivax*	*P. ovale*[b]	*P. malariae*	*P. falciparum*	*P. knowlesi*[c]	Comments
Life-threatening	Yes	No	No	Yes	Yes	
Infected RBCs	Reticulocytes	Reticulocytes	Mature erythrocytes	All	All	
Geographic region (% in area of endemicity)	Mostly in Asia (50–80)	Africa (5–8)	Global (0.5–3)	Global, most common in Africa (80–90 in Africa)	Malaysia and neighboring areas (1–60)	
Incubation period	8–17 days	10–17 days	18–40 days	8–11 days	9–12 days	May be extended for months to years
Prodromal symptoms						In all 5 species, may mimic symptoms seen with influenza or other infections; patients with early infections exhibit no periodicity and have very low parasitemias (especially immunologically naive travelers)
Severity	Mild to moderate	Mild	Mild to moderate	Mild to severe	Mild to moderate	
Initial fever pattern	Irregular (48 h)	Irregular (48 h)	Regular (72 h)	Continuous-remittent (48 h)	Very rapid (24 h)	Lack of regular periodicity may not suggest malaria as a potential diagnosis, particularly in patients who present to the emergency room early in the course of the infection
Symptom periodicity	48 h	48 h	72 h	36–48 h	24 h	*P. knowlesi* development in RBCs is asynchronous
Initial paroxysm						
Severity	Moderate to severe	Mild	Moderate to severe	Severe	Severe	
Mean duration	10 h	10 h	11 h	16–36 h	8–12 h	
Duration of untreated primary attack	3–81 wk	2–3 wk	3–24 wk	2–3 wk	2 wk	
Duration of untreated infection	5–7 yr	12 mo	20+ yr	6–17 mo	Not known; severe disease in 10%	Number of cases increasing; complications include >100,000 parasites/µl blood, acute renal impairment, jaundice, hypotension, acute/late onset respiratory distress syndrome; morphology mimics *P. malariae*; patients symptomatic at lower than sensitivity of thick film microscopy
Parasitemia limitations	<2–4%	<2%	<2%	Can be high	Can be high	*P. falciparum* and *P. knowlesi* may reach very high parasitemias; strong correlation between parasite density and development of respiratory distress
Avg/µl	20,000	9,000	6,000	20,000–500,000	600–10,000	
Max/ µl	100,000	30,000	20,000	2,000,000	236,000	
Anemia	Mild to moderate	Mild	Mild to moderate	Severe	Mild to moderate	*P. falciparum* and *P. knowlesi* can be fatal, particularly considering the rapid life cycle time for *P. knowlesi*
CNS involvement	Rare	Possible	Rare	Very common	Uncommon	No cases of cerebral involvement in *P. knowlesi*; however, infected RBCs can sequester in capillaries of brain, heart, and kidneys
Nephrotic syndrome	Possible	Rare	Very common	Rare	Common	

[a] Adapted from references 9, 10, 12, 23, 75, and 76.

[b] *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* (Fuehrer HP, Noedl H, *J Clin Microbiol* **52**:387–391, 2014).

[c] Expanded information on *P. knowlesi* from Ahmed MA, Cox-Singh J, *ISBT Sci Ser* **10**(Suppl 1):134-140, 2015.

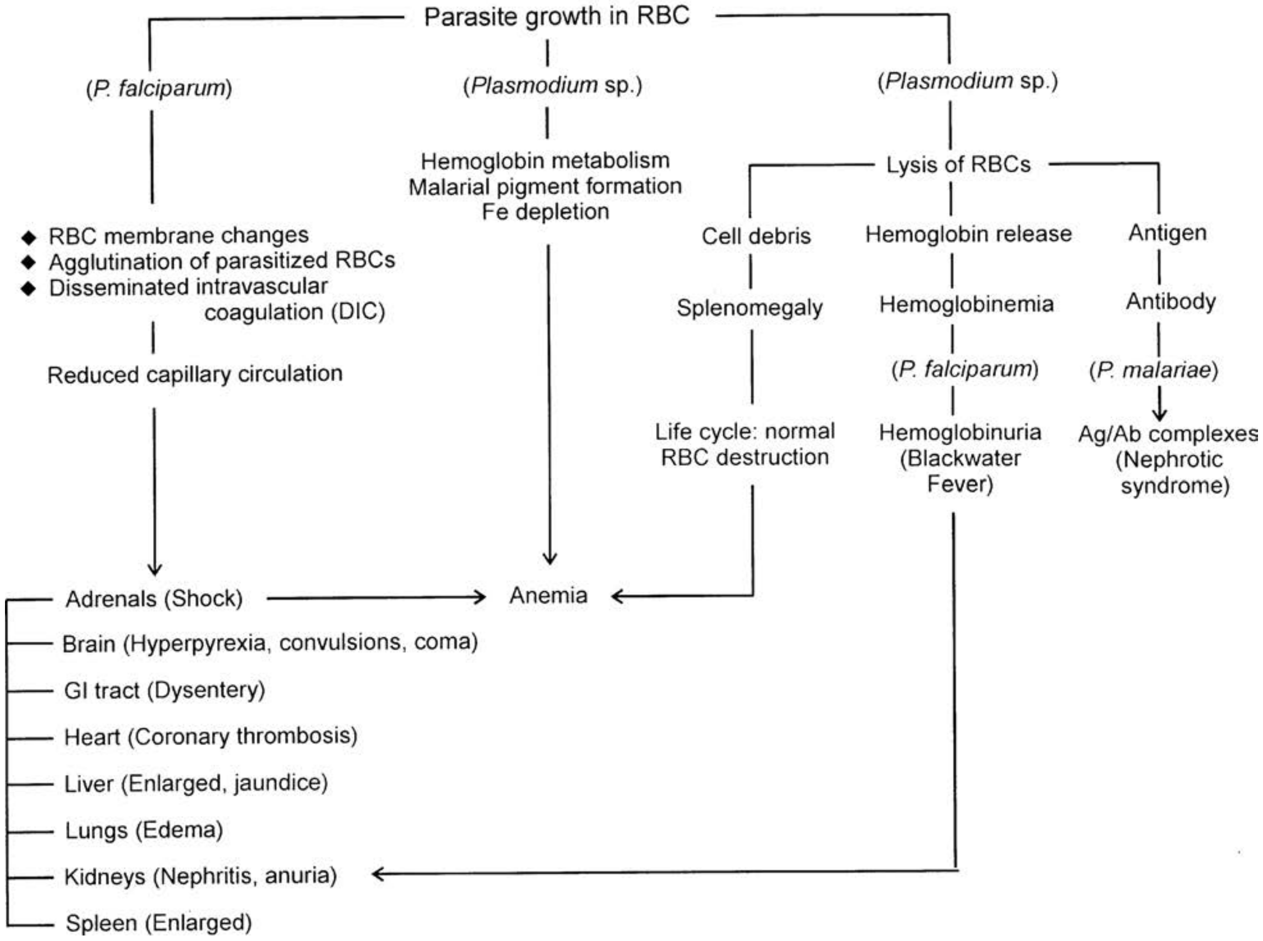

Parasite growth in RBC
(P. falciparum)
(Plasmodium sp.)
(Plasmodium sp.)
Hemoglobin metabolism
Malarial pigment formation
Fe depletion
Lysis of RBCs
◆ RBC membrane changes
◆ Agglutination of parasitized RBCs
◆ Disseminated intravascular coagulation (DIC)
Cell debris
Hemoglobin release
Antigen
Splenomegaly
Hemoglobinemia
Antibody
Reduced capillary circulation
(P. falciparum)
(P. malariae)
Life cycle: normal RBC destruction
Hemoglobinuria (Blackwater Fever)
Ag/Ab complexes (Nephrotic syndrome)
Adrenals (Shock)
Anemia
Brain (Hyperpyrexia, convulsions, coma)
GI tract (Dysentery)
Heart (Coronary thrombosis)
Liver (Enlarged, jaundice)
Lungs (Edema)
Kidneys (Nephritis, anuria)
Spleen (Enlarged)

Figure 26.8 Pathogenesis of malaria. (Adapted from Markell EK, Voge M, *Medical Parasitology*, 5th ed, WB Saunders Co, Philadelphia, PA, 1981.) doi:10.1128/9781555819002.ch26.f8

ability of the RBCs to change shape when passing through capillaries or the splenic filter may lead to plugging of the vessels.

The onset of a *P. falciparum* malaria attack occurs 8 to 12 days after infection and is preceded by 3 to 4 days of vague symptoms such as aches, pains, headache, fatigue, anorexia, or nausea. The onset is characterized by fever, a more severe headache, and nausea and vomiting, with occasional severe epigastric pain. There may be only a feeling of chilliness at the onset of fever. Periodicity of the cycle is not established during the early stages, and the presumptive diagnosis may be totally unrelated to a possible malaria infection. If the fever does develop a synchronous cycle, it is usually a cycle of somewhat less than 48 h (Tables 26.6 and 26.7, Fig. 26.13).

An untreated primary attack of *P. falciparum* malaria usually ends within 2 to 3 weeks. True relapses from the liver do not occur, and after a year, recrudescences are rare.

Severe or fatal complications of *P. falciparum* malaria can occur at any time during the infection and are related to the plugging of vessels in the internal organs, with the symptoms depending on the organ(s) involved (Table 26.3). The severity of the complications in a malaria infection may not correlate with the parasitemia seen in the peripheral blood, particularly in *P. falciparum* infections. Acute lung injury is more likely to occur in patients with very severe, multisystemic *P. falciparum* malaria than in other malaria patients. When patients present with acute lung injury and septic shock, bacterial coinfection should be suspected and treated empirically.

Disseminated intravascular coagulation is a rare complication of malaria; it is associated with a high parasite burden, pulmonary edema, rapidly developing anemia, and cerebral and renal complications. Vascular endothelial damage from endotoxins and bound parasitized blood cells may lead to clot formation in small vessels.

Cerebral malaria is most often seen in *P. falciparum* malaria, although it can occur in the other types as well.

TABLE 26.3 Malaria pathophysiology[a]

Factor	Comments[b]
Parasite virulence factors	Multiplication capacity: rapidly expanding biomass outstrips host defenses; immunity reduces multiplication RBC selectivity: age of RBC determines level of parasitemia; less so for *P. falciparum* and *P. knowlesi*, both of which can infect all ages of RBCs Cytoadherence, rosetting: all four species can induce rosetting; *P. falciparum* causes cytoadherence; possible cytoadherence also likely in *P. knowlesi* infections Cytokine release: *P. falciparum* varies in ability to induce TNF release, even those derived from single genotype; anti-inflammatory cytokines IL-1ra and IL-10 associated with *P. knowlesi*; overall cytokine levels lower than those for patients with *P. falciparum* Antigenicity: in areas of endemic infection, parasites not recognized by the host will have relative growth advantage Drug resistance: antimalarial drugs widely available, self-medication common, selective pressure against drug-sensitive parasites
Parasite multiplication	At the onset of fever, approximaely 10^8 parasites in the body; corresponds to 20–20,000 parasites/µl of blood; in areas of endemic infection, patients may tolerate parasitemias up to 10,000/µl of blood without feeling ill; *P. falciparum* and *P. knowlesi* have ability to infect all RBCs and may exceed 50%
Parasite biomass	Loose positive correlation between parasitemia and prognosis in *P. falciparum* and *P. knowlesi* malaria; positive correlation between number of sequestered parasitized RBCs and severity of *P. falciparum* malaria
Toxicity and cytokines	Question of true toxin versus paroxysmal cytokine release due to nonspecific reaction to amount of particulate debris released into the circulation at schizont rupture; high concentrations of cytokines appear to be harmful, while lower levels probably benefit the patient; they are responsible for the typical symptoms of fever and malaise
RBC deformability	As the parasite grows, the normally flexible biconcave disk becomes more spherical and rigid, and the surface becomes irregular and covered with small protrusions; reduction in deformability results from reduced membrane fluidity, increasing sphericity, and enlarging, viscous intraerythrocytic parasite; also occurs in uninfected RBCs in acute malaria
Sequestration and pathology	RBCs adhere to microvascular endothelium (cytoadherence) and disappear from peripheral circulation (i.e., sequestration); greatest involvement is in brain, heart, kidney, intestines, and adipose tissue, least prominent is in skin; consequences of microcirculatory obstruction are reduced oxygen/substrate supply (anaerobic glycolysis and lactic acidosis)
Coma	Coma associated with 15–20% mortality; however, both adults and children recover without sequelae; thought to be metabolic or anesthetic encephalopathy (may involve neurotransmitter abnormalities and NO)
Intracranial pressure	Mild generalized increase in systemic vascular permeability in severe malaria; majority of patients with cerebral malaria do not have cerebral edema; all children with cerebral malaria (at some time) have elevated ICP; background rise in ICP probably results from increase in cerebral blood volume, independent of permeability
Immunological factors	Unlikely that cerebral malaria is due to immune-mediated damage; fatal falciparum malaria has lack of extravascular pathology; in cases of hypergammaglobulinemia, most antibody is not directed against malaria antigens; description suggests a "smoke screen" with broad-spectrum and nonspecific activation that interferes with the development of a specific cellular immune response; exception may be nephrotic syndrome caused by *P. malariae* and antigen-antibody immune complex formation in the kidneys
Renal failure	Basic pathology is acute tubular necrosis; urine sediment unremarkable, sodium level in urine low, blood pressure normal; no proof, but renal microvascular obstruction due to sequestration in kidneys may be responsible; glomerulonephritis is very rare in acute malaria, and renal necrosis does not occur; renal complications more common with *P. malariae* and *P. knowlesi*
Pulmonary edema	Pulmonary function usually normal; pregnant women with severe falciparum malaria at risk for sudden, acute pulmonary edema (resulting from a sudden increase in pulmonary capillary permeability); cause not known
Respiratory distress syndrome	Acute and late-onset respiratory distress syndrome seen; included in WHO guidelines for severe malaria in the nonimmune (immunologically naive, no prior exposure to malaria), *P. falciparum*, *P. knowlesi*
Cardiovascular function	Pump function not a problem in severe malaria; may be autonomic dysfunction in acute malaria (postural hypotension associated with impaired cardioacceleration), but may be common to all febrile infections

(continued)

TABLE 26.3 Malaria pathophysiology[a] *(continued)*

Factor	Comments[b]
Fluid space; electrolyte changes	Patients may be dehydrated; mild hyponatremia and hypochloremia are common, but potassium levels are usually normal (in spite of hemolysis); after rehydration, total body water and extracellular volume are normal
Anemia	In general, degree of anemia corresponds to duration and severity of infection; obligatory destruction of RBCs due to life cycle; accelerated destruction of nonparasitized RBCs correlates with disease severity; condition is compounded by bone marrow dysfunction; factors that trigger removal of nonparasitized RBCs not fully explained; RBC survival reduced in malaria (not affected by corticosteroids); reduced oxygen carriage; past explanation of death due to congestive cardiac failure may be due to tissue hypoxia and severe metabolic acidosis
Coagulopathy and thrombocytopenia	Accelerated coagulation cascade activity with accelerated fibrinogen turnover; moderate thrombocytopenia common in *P. falciparum* and *P. vivax* malaria, caused by splenic clearance; DIC no longer considered important in pathogenesis of malaria
Blackwater fever	Massive intravascular hemolysis and passage of dark red, brown, or usually black urine; occurs when patients with G6PD deficiency take oxidant drugs (whether or not they have malaria), when G6PD-deficient patients have malaria and receive treatment with quinine or artemisinin derivatives, and when some patients with normal G6PD levels are treated with quinine or artemisinin derivatives; blackwater fever is not usually accompanied by renal failure, although renal impairment is common; can be seen with all species (more severe with *P. falciparum*)
Spleen	Rapid splenic enlargement for removal of parasitized RBCs; may also modulate cytoadherence, but mechanism is not known
Gastrointestinal tract	Minor ulceration of stomach and duodenum common in severe malaria; may be reduced splanchnic perfusion (malabsorption of sugars, fats, and amino acids); may be increased gut permeability
Liver	Hepatic dysfunction usually seen in severe malaria; jaundice common (hemolytic, hepatic, cholestatic components); reduced clotting factor synthesis, metabolic drug clearance, and biliary excretion; failure of glyconeogenesis (lactic acidosis and hypoglycemia); liver blood flow may be reduced or may be elevated
Acidosis	Consistent feature; arterial, capillary, venous, and CSF lactate concentrations rise in direct proportion to disease severity; majority of lactic acid produced in malaria derives from host rather than parasite; also feature of severe *P. knowlesi* infection
Hypoglycemia	Closely associated with hyperlactatemia; hypoglycemia leads to nervous system dysfunction and is associated with residual neurological deficit in survivors of cerebral malaria; does not tend to be an issue with *P. knowlesi* malaria
Placenta	Pregnancy increases susceptibility to malaria (suppression of systemic and placental cell-mediated immune responses); intense sequestration of *P. falciparum*-infected RBCs in placenta and maternal anemia; leads to placental insufficiency and fetal retarded growth; maternal mortality approaches 50%, and fetal death usual; high incidence of cerebral malaria, hypoglycemia, and pulmonary edema; *P. vivax* also linked to reductions in birth weight, suggesting that systemic effects of malaria may be more important than previously recognized
Coincident infections	Patients vulnerable to other infections, including other species of malaria; relationship to HIV also questionable; chronic hepatitis B virus carriage also associated with increased risk of severe malaria

[a] Adapted from references 2, 9, 10, 12, 23, and 75–78.

[b] CSF, cerebrospinal fluid; DIC, disseminated intravasular coagulopathy; G6PD, glucose-6-phosphate dehydrogenase; ICP, intracranial pressure; IL, interleukin; NO, nitric oxide; TNF, tumor necrosis factor.

If the onset is gradual, the patient may become disoriented or violent or may develop severe headaches and pass into coma. Some patients, even those who exhibit no prior symptoms, may suddenly become comatose. Physical signs of central nervous system (CNS) involvement are quite variable, and there is no real correlation between the severity of the symptoms and the peripheral blood parasitemia. It has been shown that patients with cerebral malaria were infected with RBC rosette-forming *P. falciparum* and that plasma from these patients generally had no antirosetting activity. A rosette usually consists of a parasitized RBC surrounded by three or more uninfected RBCs (Fig. 26.14A). Interaction with adjacent uninfected RBCs in rosettes appears to be mediated by knobs seen on the parasitized RBC (Fig. 26.14B) (14). In contrast, *P. falciparum* parasites from patients with mild malaria lacked the rosetting phenotype or had a much lower rosetting rate. Also, antirosetting activity has been detected in the plasma of these patients. These findings strongly support the idea that RBC rosetting contributes to the pathogenesis of cerebral malaria while antirosetting antibodies offer protection against these clinical sequelae.

Interplay among many factors, including free oxygen radicals and other mediators of inflammation, is likely to

TABLE 26.4 *Plasmodium* pathogenesis and sequelae

Clinical situation and condition	Comments[a]	Reference(s)
Chronic malaria in West Africa	West Africa; SLVL and hyperreactive malarial splenomegaly have clinical and immunological features in common. Dysregulated immune response to repeated malaria infections results in a stimulated, proliferating pool of B cells; environmental and other factors promote the development of SLVL. Several infectious agents have been associated with B-cell non-Hodgkin's lymphomas.	79
Clinically severe malaria; 489 patients in Zimbabwe	Malaria and blood group A patients had lower hemoglobin levels; also at higher risk of coma than patients in other blood groups	80
64 *P. falciparum* patients in eastern Sudan; plasma samples and seasonal agglutination of infected RBCs	First demonstration of marked seasonal fluctuations in capacity of sera to agglutinate parasitized RBCs; may reflect drop in levels of agglutinating antibodies between seasons or shifts in antigens being recognized from one transmitting season to the next	81
Cerebral malaria (*P. falciparum*); 250,000 Vietnam veterans	CM results in multiple, major, substantially underappreciated neuropsychiatric symptoms in Vietnam veterans (poor dichotic listening, "personality changes," depression, and, in some cases, partial seizure-like symptoms). History of malaria needs to be assessed in workup of any Vietnam veteran; positive response could dramatically change diagnosis and therapy. VEGF and its receptor-related molecules are overexpressed in the brain tissues of CM patients. Factors associated with CM are (from most important): pulmonary edema, splenomegaly, fever, high parasitemias, dyspnea, hepatomegaly, higher systolic blood pressure, higher body mass index.	82–84
Bone marrow transplantation in recipient of multiple blood transfusions	*P. falciparum* trophozoites and gametocytes found on peripheral blood smears	85
Problem with donors living in high-risk areas	11/420 patients (2.6%) positive for *P. falciparum* (mean time 22 + 44 days)	86
Both donor and recipient checked prior to BMT	Recommendation for treatment prior to harvest and conditioning of cells, regardless of smear results; patient infection with *P. falciparum* 11 days after stem cell transplant; critical to check donor/recipient in areas of endemicity	87, 88
Case of unrelated donor	Case of *P. vivax* infection, donor asymptomatic 11 months prior to donation	89
Relapse of primary unidentified infection	Case of *P. vivax* infection after BMT	90
Case of pancytopenia after 70 days	Case of *P. vivax* infection after BMT; always consider malaria in the differential	91
Severe *P. falciparum* malaria; 24 adults (NO)	No support for the hypothesis that excessive NO production contributes to the pathogenesis of severe falciparum malaria. However, local changes in NO production (CNS) might not be reflected in total production or levels in the CSF.	92, 93
NO production varies throughout childhood (African children)	NO levels high in infancy, decrease after first year of life, then increase again after 5 yr of age. Elevated NO in both infants and older children may be related to age and malaria infection, respectively, and may be one of the mediators of anti-disease immunity in these two age groups.	94
NO production related to anemia (Tanzania)	NO production was inversely associated with hemoglobin concentration; thus, it is not a predictor of anemia after correction of age/gender data.	95
Cerebral malaria (*P. falciparum*)	Identification of rare *P. falciparum* parasites expressing a panadhesive phenotype linked to RBC rosetting (correlate of CM)	96
	Parasitized RBCs may bind integrin $\alpha_v\beta_3$ on microvascular endothelial cells; sequestration leads to cerebral malaria.	97
	Binding of parasitized RBCs to cerebral endothelial cells leads to functional changes in the blood-brain barrier in CM.	98
Adhesion phenomena (*P. falciparum*)	Cytoadherence begins at 12 h and peaks at 28 h; rosette formation (16–20 h); rosette formation and immunoglobulin binding rate of about 50% (24–36 h)	99
Molecular mechanisms of cytoadherence (*P. falciparum*)	Cytoadherence may activate intracellular signaling pathways in both endothelial cells and infected RBCs, leading to gene expression of mediators such as cytokines, which could modify the outcome of the infection.	100, 101
RBC deformability (*P. falciparum*)	Reduced deformability of uninfected RBCs at high shear stresses and subsequent splenic removal of these cells may be an important contributor to anemia of severe malaria.	92

(continued)

TABLE 26.4 *Plasmodium* pathogenesis and sequelae *(continued)*

Clinical situation and condition	Comments[a]	Reference(s)
Eosinophil activity (*P. falciparum*)	Appears that low eosinophil counts may be due to tissue sequestration and destruction rather than decreased production; levels of eosinophilia granule proteins in plasma correlated with levels of TNF and soluble IL-2 receptor, implicating inflammatory responses and T-cell activation as causes of eosinophil activation	102
Fatal malaria (*P. falciparum*) in African children	Three clinical syndromes: severe anemia, CM, malaria-associated hyperapnea (increased rate and depth of breathing); "respiratory distress" may be misleading, malaria hyperapneic syndrome (severe systemic acidosis) may not be recognized in conscious children	103
Frequent parasitemias with *P. falciparum* (asymptomatic); children in Tanzania	Asymptomatic (especially polyclonal) *P. falciparum* infection protects against clinical disease due to new parasites.	104
Maternal and infant mortality (*P. falciparum*)	Accumulation of large numbers of infected RBCs in maternal blood spaces of placenta may be mediated by adhesion of infected RBCs to molecules on the syncytiotrophoblast surface. Parasites infecting pregnant women may be antigenically distinct from those common in childhood disease.	105
P. vivax malaria	Although *P. vivax* is seldom fatal, it is a major cause of morbidity. *P. vivax*-infected RBCs form rosettes like those seen in *P. falciparum* infections.	9, 106
P. malariae malaria	Blood stage parasites may persist for the life of the host; immune complexes in kidneys may cause structural glomerular damage.	12
P. ovale malaria	Two types of *P. ovale* based on nucleotide deletions and substitutions in the 18S rRNA gene; these parasites have been found to coexist in Vietnam, Thailand, and Myanmar; generally least severe of 5 human species of malaria	10
P. knowlesi malaria	Endemic in Malaysian Borneo; very short life cycle (24 h); early infection mimics *P. falciparum*, older stages mimic *P. malariae*; can infect all ages of RBCs with high parasitemias like those seen in *P. falciparum* malaria; can result in fatal outcome	75

[a] BMT, bone marrow transplantation; CM, cerebral malaria; CSF, cerebrospinal fluid; IL-2, interleukin-2; NO, nitric oxide; SLVL, splenic lymphoma with villous lymphocytes; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

be involved in the pathogenesis of cerebral malaria (2, 15). Sequestration often predisposes to cerebral symptoms but in ways not necessarily restricted to obstruction of blood flow. Tumor necrosis factor alpha and other pyrogenic cytokines play a role in the regulation of parasite density in the host; cerebral malaria is also thought to be related to excessive tumor necrosis factor alpha.

Magnetic resonance imaging of the brains of patients with cerebral malaria shows that the volume of the brain is increased. This increased volume probably results from sequestration of parasitized RBCs and compensatory vasodilatation rather than from edema. Brain stem herniation may occur, but its temporal relation to brain death in patients with cerebral malaria is still unclear. Ultrastructural study of the brain in patients with fatal *P. falciparum* infections reveals that sequestration of parasitized RBCs in cerebral microvessels is much higher in the brains of patients with cerebral malaria (cerebrum, cerebellum, and medulla oblongata) than in the brains of patients with noncerebral malaria. There were 26.6 times more parasitized RBCs in the brain microvasculature than in the peripheral blood (2).

Extreme fevers, 107°F (ca. 41.7°C) or higher, may develop in a relatively uncomplicated attack of malaria or may develop as another manifestation of cerebral malaria. Without vigorous therapy, the patient usually dies. Cerebral malaria is considered to be the most serious complication and the major cause of death from infection with *P. falciparum*; this complication accounts for up to 10% of all *P. falciparum*-infected patients admitted to the hospital and for 80% of fatal cases.

Bilious remittent fever involves the liver, with symptoms including abdominal pain, nausea, and severe and persistent vomiting, with the vomitus containing evidence of bile or fresh blood. There may also be severe diarrhea or dysentery with resulting dehydration. The liver is large and tender, the skin becomes icteric, and the urine contains bile.

Diarrhea or dysentery without liver involvement or jaundice is also seen, with malabsorption characterized by decreased absorption of D-xylose and vitamin B_{12} and a low carotene level. Jejunal biopsy reveals edema, round cell infiltration of the lamina propria, and blunting of villi, which have also been found on postmortem examinations.

Algid malaria symptoms include circulatory collapse; low blood pressure; hypothermia; rapid, thready pulse; and pale, cold, clammy skin. There may be severe abdominal pain, vomiting and diarrhea, and muscular cramps. At postmortem examination, the adrenal glands are congested, necrotic, and hemorrhagic. In the subacute stage

TABLE 26.5 Immunopathology of malaria[a]

Pathology	Identified or possible mechanisms[b]
Autoimmunity	Autoantibodies Antinuclear antibodies
Immunosuppression	Antigenic competition Decreased lymphocyte production Disruption of spleen function Macrophage dysfunction Polyclonal activation and immune system "exhaustion"
Hypergammaglobulinemia	Ag-induced cytokine production (IL-6)
Anemia	Anti-RBC antibodies Decrease in RBC production (effect of TNF) Increased RBC destruction Iron deficiency
Thrombocytopenia	Increased platelet removal Coating of platelets with malarial antigen; subsequent platelet destruction
Hyperactive malarial splenomegaly	Genetic predisposition Hypogammaglobulinemia Chronic increase of lymphocyte proliferation
Nephrotic syndrome	Immune complex (Ag-Ab) deposition on kidney glomeruli Autoimmunity

[a] Adapted from references 78, 107, and 108.
[b] Ab, antibody; Ag, antigen; IL, interleukin; TNF, tumor necrosis factor.

and after recovery, some patients develop signs resembling Addison's disease.

Although infrequent, blackwater fever is most often associated with *P. falciparum* malaria. Usually, there is a history of previous malarial attacks. Sudden, intravascular hemolysis results in a dramatic color change in the urine (acidic urine with a high methemoglobin content). Since the hemoglobinuria does not necessarily give rise to symptoms, the onset may be missed. Quinine sensitivity has been postulated as one possible factor leading to blackwater fever. Another has been the presence of antibodies acting as hemolysins against RBC antigens. The parasitemia may be relatively low in patients with blackwater fever. With the increased use of quinine to treat chloroquine-resistant patients who live in areas with malaria, more cases of blackwater fever may be seen in the future.

Acute renal failure may also occur unassociated with blackwater fever and hemolytic anemia. Renal anoxia is thought to lead to tubular necrosis. The nephrotic syndrome, caused by acute glomerulonephritis, has also been found in some patients with *P. falciparum* malaria.

Independent risk factors contributing to anemia in falciparum malaria in patients presenting with anemia on admission include age younger than 5 years, a palpable spleen, a palpable liver, recrudescent infections, being female, a history of prolonged history of illness (>2 days) before admission, and pure *P. falciparum* infections rather than mixed infections with *P. falciparum* and *P. vivax*. It appears that coinfection with *P. vivax* can modify the severity of the disease.

TABLE 26.6 Parasitemia determined from conventional light microscopy: clinical correlation[a]

Parasitemia (%)	No. of parasites/µl	Clinical correlation[b]
0.0001–0.0004	5–20	Number of organisms that are required for a positive TBF (sensitivity) **Note (TBF):** Examination of 100 TBF fields (0.25 µl) may miss up to 20% of infections (sensitivity, 80–90%); at least 300 TBF fields should be examined before reporting a negative result **Note (THBF):** Examination of 100 THBF fields (0.005 µl); at least 300 THBF fields should be examined before reporting a negative result; *both* TBF and THBF should be examined for every specimen submitted for a suspect malaria case. **One set (TBF + THBF) of negative blood films does not rule out a malaria infection.**
0.002	100	Naive patients may be symptomatic below this level; remember emergency room patients and travelers.
0.1	5,000	BinaxNOW (maximum sensitivity); patients with low parasitemias will often have a false-negative rapid test; if negative, then STAT thick and thin films are required.
0.2	10,000	Level above which immune patients will exhibit symptoms
2	100,000	Maximum parasitemia of *P. vivax* and *P. ovale* (infect young RBCs only) (rarely may exceed 2%)
2–5	100,000–250,000	Hyperparasitemia, severe malaria,[c] increased mortality
10	500,000	Exchange transfusion may be considered, high mortality

[a] Adapted from references 23 and 109.
[b] TBF, thick blood film; THBF, thin blood film.
[c] WHO criteria for severe malaria are parasitemia of >10,000/µl and severe anemia (hemoglobin, <5 g/liter). Prognosis is poor if >20% of parasites are pigment-containing trophozoites and schizonts and/or if >5% of neutrophils contain visible pigment.

TABLE 26.7 Malaria characteristics

Organism	Characteristics
Plasmodium vivax (benign tertian malaria)	48-h cycle Tends to infect young cells (reticulocytes) Enlarged RBCs Schüffner's dots (true stippling) after 8–10 h Delicate ring; occasionally may see 2 rings/cell (like *P. falciparum*) Very ameboid trophozoite Mature schizont contains 12–24 merozoites
Plasmodium malariae (quartan malaria)	72-h cycle (long incubation period) Tends to infect old, mature cells Normal-size to small RBCs No stippling Thick ring, large nucleus Trophozoite tends to form "bands" across the cell Mature schizont contains 6–12 merozoites (rosette or "daisy")
Plasmodium ovale	48-h cycle Tends to infect young cells (reticulocytes) Enlarged RBCs (may or may not always be oval) with fimbriated edges Schüffner's dots appear in the beginning (in RBCs with very young ring forms, in contrast to *P. vivax*) Smaller ring than *P. vivax* Trophozoite less ameboid than that of *P. vivax* Mature schizont contains average of 8 merozoites
Plasmodium falciparum (malignant tertian malaria)	36–48-h cycle Tends to infect any cell regardless of age; thus, very heavy infection may result All sizes of RBCs No Schüffner's dots (Maurer's dots: may be larger, bluish, single dots) Multiple rings/cell (only young rings, gametocytes, and occasional mature schizonts seen in peripheral blood) (in severe, very high parasitemia cases, other blood stages may be seen) Delicate rings; may have 2 dots of chromatin per ring ("headphones"), appliqué or accolé forms Crescent-shaped gametocytes; takes approximately 10 days to appear
Plasmodium knowlesi (simian malaria)	~24-h cycle Tends to infect any cell regardless of age; thus, very heavy infection may result All sizes of RBCs, but most tend to be normal size No Schüffner's dots (faint, clumpy dots later in cycle) Multiple rings/cell (may have 2–3) Delicate rings; may have two dots of chromatin/ring ("headphones"), appliqué or accolé forms Band form trophozoites commonly seen Mature schizont contains 16 merozoites, no rosettes Gametocytes round, tend to fill the cell **Note:** Early stages mimic *P. falciparum* ring forms; later stages mimic *P. malariae* band forms, etc. (often misdiagnosed as a *P. falciparum/P. malariae* mixed infection)

In African children with malaria, the presence of impaired consciousness or respiratory distress can identify those at high risk of death. Biochemical evidence of hepatic and renal dysfunction also suggests a poor prognosis; however, in contrast to adults with malaria, children with severe malaria do not always have acute renal failure.

Using several different approaches, it is estimated that approximately 100,000 infant deaths in Africa could be attributed to malaria during pregnancy, resulting in low-birth-weight babies. Antimalarial intermittent preventive treatment during pregnancy has been estimated to prevent the death of 22,000 children younger than 5 years through reduced numbers of preterm deliveries. However, if one assumes an efficacy rate of 80% in preventing placental malaria, the number of deaths prevented may be closer to 80,000.

Plasmodium knowlesi**.** In addition to the Kapit Division of Sarawak in Malaysian Borneo, there have also been reports of locally acquired *P. knowlesi* infections from Southern Thailand, the Myanmar-China border, the Philippines, and Singapore, indicating that transmission occurs in many Southeast Asian countries (Fig. 26.15). *P. knowlesi* is primarily a chronic infection of the long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques (Fig. 26.16). The early stages are easily confused with *P. falci-*

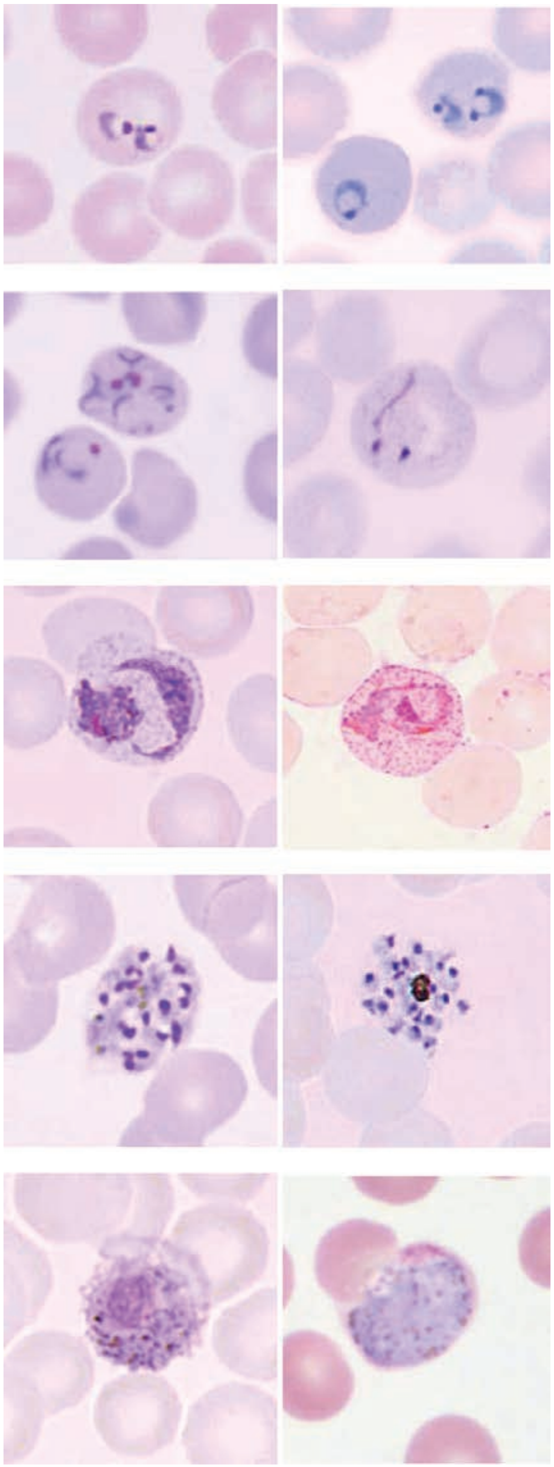


Figure 26.9 *Plasmodium vivax*. (Top row) Ring forms (note that double rings are not limited to *P. falciparum* and that the RBC is beginning to enlarge as the rings grow). (Row 2) Early trophozoites. (Row 3) Developing trophozoites (note the ameboid nature of the trophozoites and the presence of Schüffner's dots). (Row 4) Developing schizonts (note the number of merozoites). (Bottom row) Male and female gametocytes.
doi:10.1128/9781555819002.ch26.f9

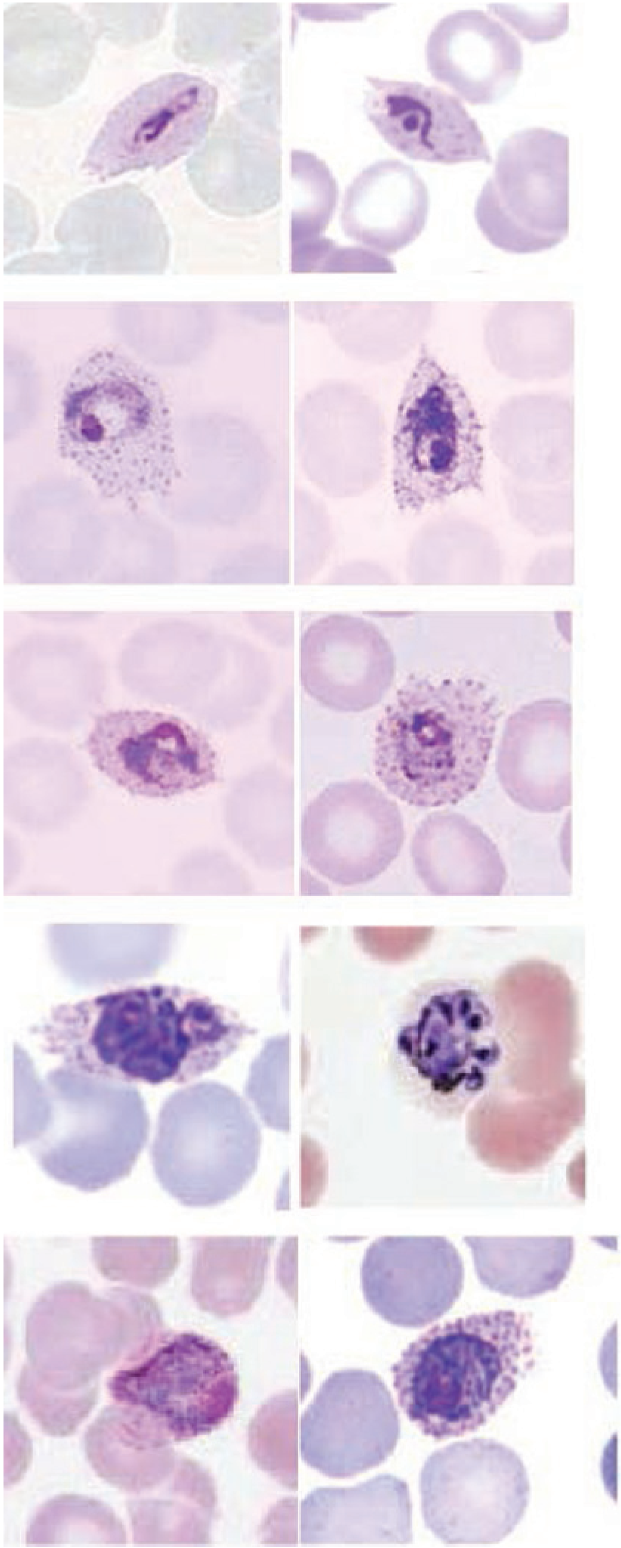


Figure 26.10 *Plasmodium ovale*. (Top three rows) Developing trophozoites (note the nonameboid trophozoites, presence of Schüffner's dots, oval RBCs, and fimbriated edges of infected red cells). (Row 4) Developing schizonts. (Bottom row) Gametocytes.
doi:10.1128/9781555819002.ch26.f10

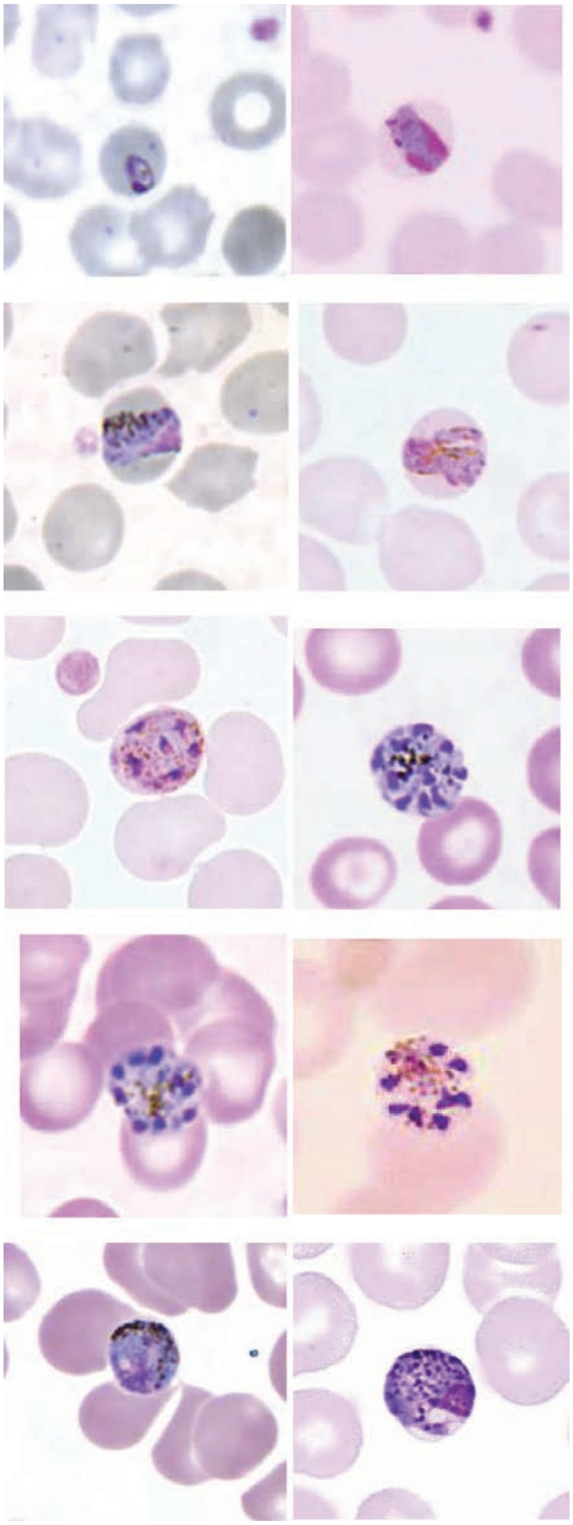


Figure 26.11 *Plasmodium malariae*. (Top and row 2) Early ring form and band form developing trophozoites (note that the RBC is normal or slightly smaller than normal). (Row 3) Developing schizonts (note the small number of merozoites). (Row 4) Mature schizonts. (Bottom row) Gametocytes.
doi:10.1128/9781555819002.ch26.f11

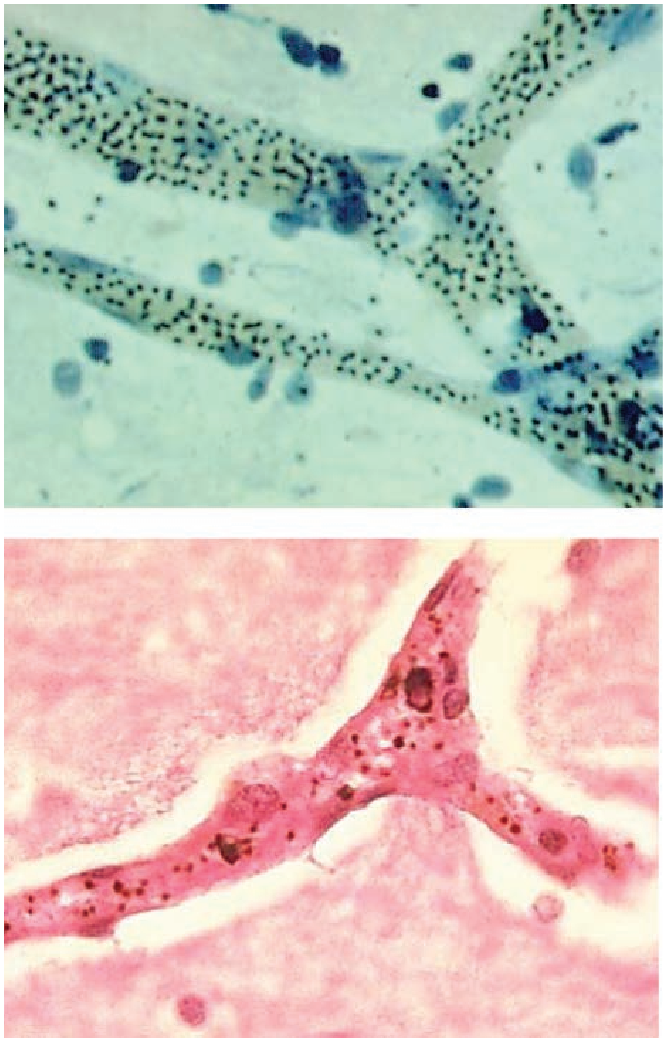


Figure 26.12 *Plasmodium falciparum*-parasitized blood cells plugging the capillaries in brain tissue; note the dark malarial pigment. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch26.f12

parum, while the later stages mimic *P. malariae* on blood film microscopy in cases of human infection. The erythrocyte invasion by *P. knowlesi* is not limited to young or old cells, which allows high parasitemia, and development of the parasite in erythrocytes is asynchronous (Fig. 26.17, Tables 26.6 and 26.7).

P. knowlesi is relatively closely related with *P. vivax*; therefore, it is not surprising that infections with these two species share some common features, such as occasional severity and marked thrombocytopenia. However, disease caused by *P. knowlesi* is somewhat more severe than disease caused by *P. vivax*, although vivax malaria can also be severe in a significant number of cases, at least in certain regions (severe malaria in ~3%, compared with 7 to 10% in individuals with *P. knowlesi* malaria).

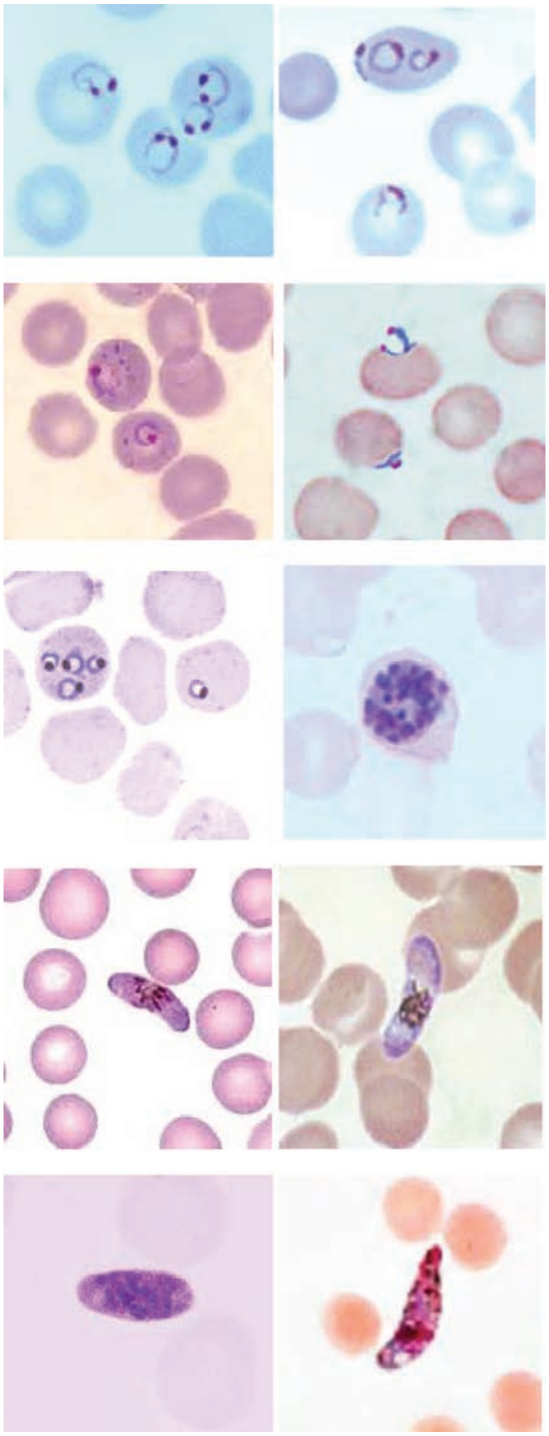


Figure 26.13 *Plasmodium falciparum*. (Top and row 2) *P. falciparum* ring forms. Note the double rings per cell, appliqué or accolé form, and headphone appearance of some rings; also note Maurer's clefts in the RBCs (row 2, left). (Row 3) Ring forms and mature schizont (rarely seen in blood films). (Row 4) Crescent-shaped gametocytes. (Bottom, left) Immature gametocyte; (right) ookinete from mosquito cycle (found in thin blood film from EDTA-treated blood with lag time prior to film preparation). doi:10.1128/9781555819002.ch26.f13

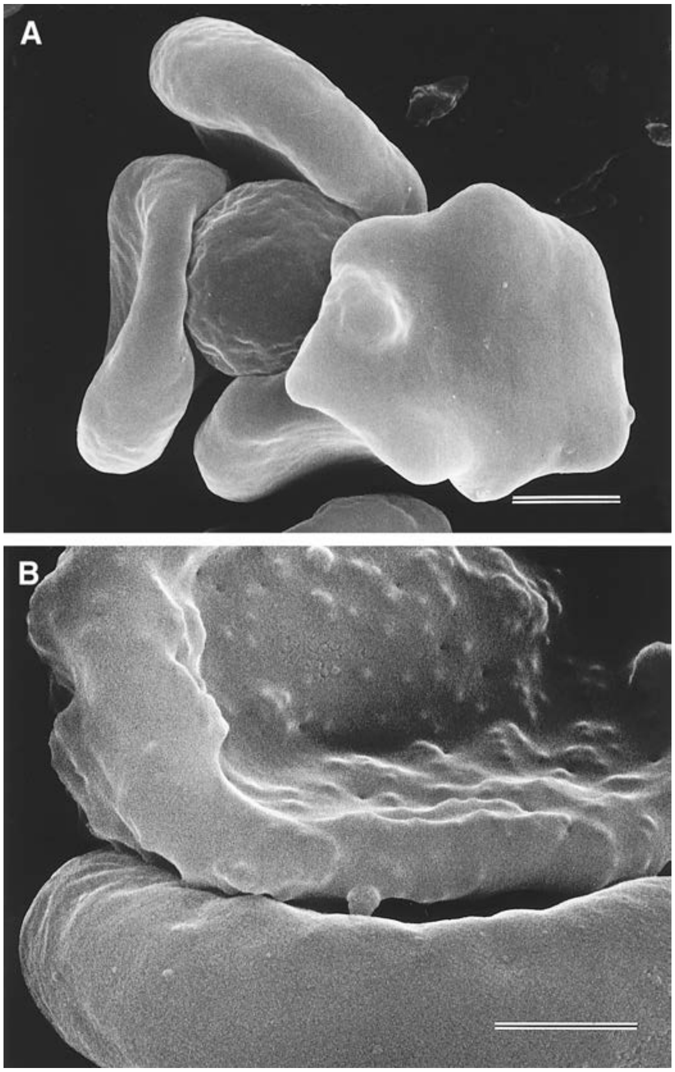


Figure 26.14 (A) Scanning electron micrograph of a rosette consisting of a central parasitized RBC surrounded by several attached uninfected RBCs (original magnification, ×19,000; bar, 1 µm). (B) Scanning electron micrograph of the interaction of a parasitized RBC with an uninfected RBC that appears to be mediated by protrusion of knobs (original magnification, ×53,000; bar, 0.5 µm). (From reference 101 with permission.) doi:10.1128/9781555819002.ch26.f14

In Malaysia, the patients with *P. knowlesi* infection typically present with a febrile illness with daily fever and chills. Other frequent symptoms included headache, rigors, malaise, myalgia, abdominal pain, breathlessness, and productive cough. Tachypnea, pyrexia, and tachycardia were common clinical signs. Although the patients had thrombocytopenia at hospital admission or on the following day, none had clinical coagulopathy. At hospital admission, only a few of the patients had anemia, whereas mild hepatic

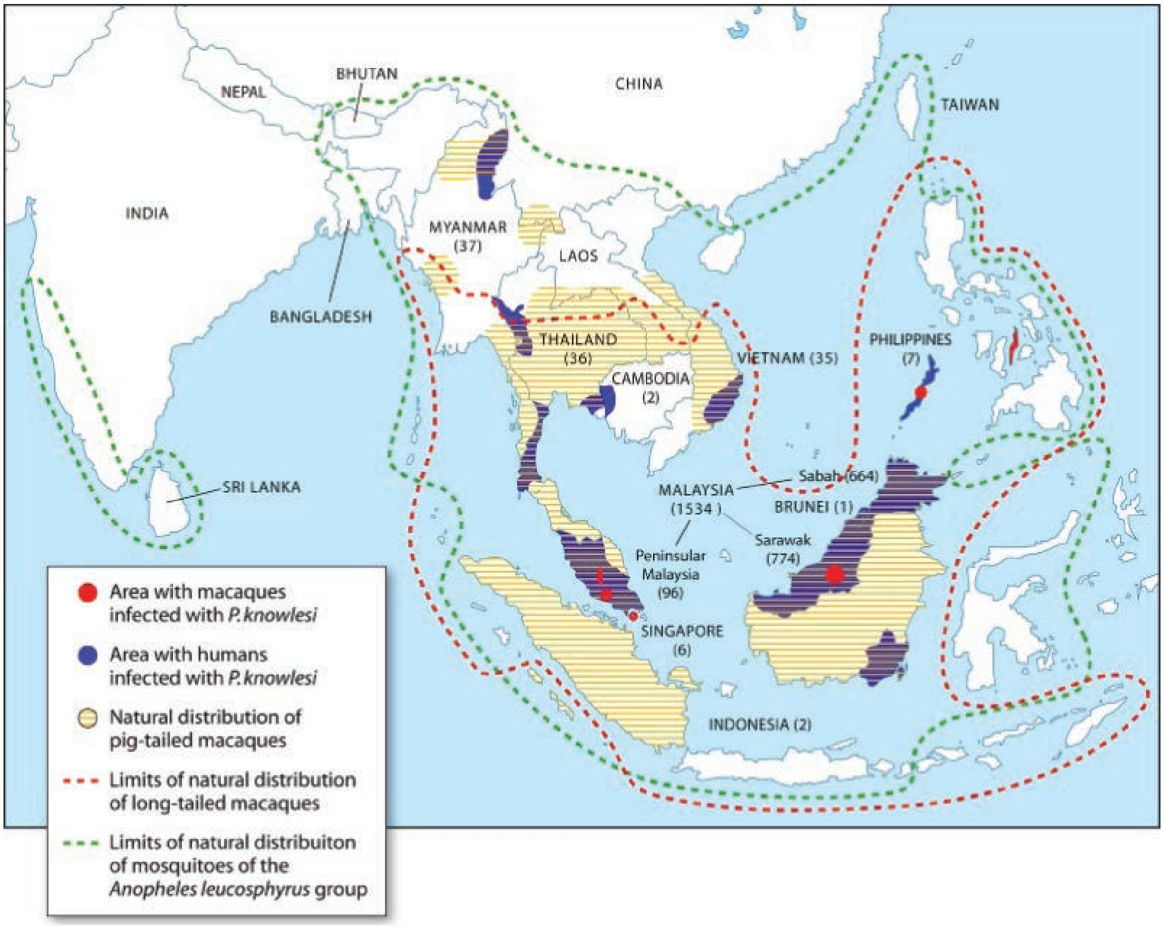

BHUTAN
NEPAL
CHINA
TAIWAN
INDIA
MYANMAR (37)
LAOS
BANGLADESH
THAILAND (36)
CAMBODIA (2)
VIETNAM (35)
PHILIPPINES (7)
SRI LANKA
MALAYSIA (1534)
Sabah (664)
BRUNEI (1)
Sarawak (774)
Peninsular Malaysia (96)
SINGAPORE (6)
INDONESIA (2)
● Area with macaques infected with *P. knowlesi*
● Area with humans infected with *P. knowlesi*
○ Natural distribution of pig-tailed macaques
- - - Limits of natural distribution of long-tailed macaques
- - - Limits of natural distribuiton of mosquitoes of the *Anopheles leucosphyrus* group

Figure 26.15 *Plasmodium knowlesi* infections reported in humans and macaques and limits of natural distribution of mosquito vectors and of macaques. The numbers in parentheses represent numbers of *P. knowlesi* cases reported for each Southeast Asian country or region in Malaysia. (Courtesy of reference 75.) doi:10.1128/9781555819002.ch26.f15

dysfunction was relatively common. The great majority of patients (94%) experienced no complications, and the infection responded well to chloroquine and primaquine treatment (16–18). In patients with severe infections, the most frequent complication was respiratory distress.

P. knowlesi is unique among the primate and human malarias in that it has a 24-h erythrocytic cycle, which tends to accelerate the development of complications. Information on the characteristics of knowlesi malaria in humans indicates that the levels of parasitemia vary over a wide range among different patients. The *P. knowlesi* parasitemia at hospital admission tends to be strongly and independently associated with renal dysfunction; renal failure is seen despite interventions. Unlike serious cases of *P. falciparum*, neurological symptoms with *P. knowlesi* are rare, and no cases of cerebral malaria have been reported (18). However, autopsy findings in a fatal case of *P. knowlesi* malaria indicate that *P. knowlesi*-infected red cells can sequester in capillaries of the brain, heart, and kidneys. In some of the fatal cases, a high parasitemia was seen (15%, >10%, and >10 parasites per high-power microscope field).

Since *P. knowlesi* malaria can progress rapidly to severe disease, these presumptive cases should be treated like *P. falciparum* malaria if the species identification is based on microscopic examination alone or if coinfection with *P. falciparum* cannot be excluded with certainty using PCR. It is important to remember that diagnostic results should include a comment: "Unable to rule out *P. falciparum* or *P. knowlesi*." This information alerts the physician to the possibility of severe disease and possible fatal outcomes. This approach is particularly important if the microscopic examination of blood films suggests *P. malariae* but the patient has severe disease, a high parasitemia (>0.1%), or a recent history of visiting woods or their vicinity in Southeast Asia. Unfortunately, neither the pLDH- nor aldolase-based rapid tests demonstrate sufficiently high overall sensitivity for *P. knowlesi*. More sensitive rapids are

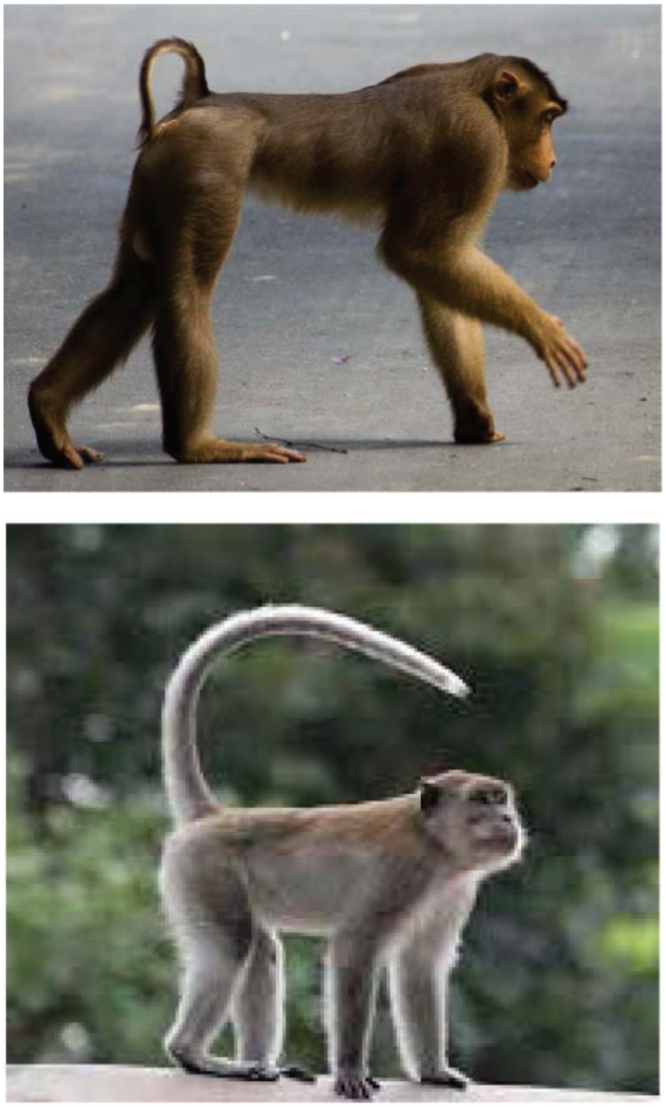


Figure 26.16 *Plasmodium knowlesi* (simian malaria). (Upper) Pig-tailed macaque (courtesy of https://www.flickr.com/photos/erwinb/2986619018/sizes/o/in/photostream/). (Lower) Long-tailed macaque (courtesy of http://www.ecosystem-guides.com/Bukit-Lawang.html). doi:10.1128/9781555819002.ch26.f16

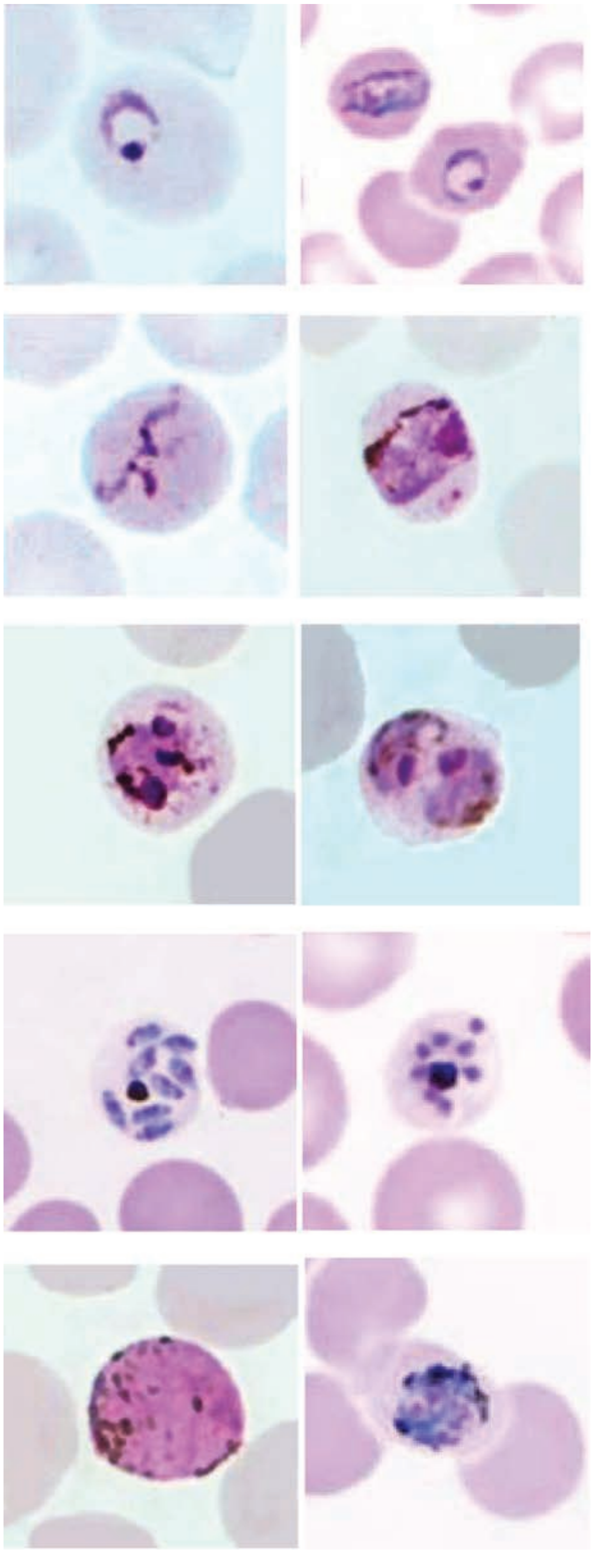


Figure 26.17 *Plasmodium knowlesi*. (Top three rows) Developing trophozoites (note the band forms which can mimic *P. malariae*; ring forms can mimic *P. falciparum*). (Row 4) Mature schizonts. (Bottom) Gametocytes. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch26.f17

needed in regions of *P. knowlesi* endemicity; new test development is currently under way.

Mixed Infections. Coincident infection with more than one species of malaria is more common than previously suspected. As an example, in Thailand, up to 30% of patients who present with severe *P. falciparum* malaria are also infected with *P. vivax*. Based on overlapping geographic areas where both *P. falciparum* and *P. vivax* are found, it is not surprising that dual infections occur. In Africa, dual infections with *P. falciparum* and *P. malariae* have also been found. Although the figures may be underestimates, in Gambian children the prevalence of mixed-species infection varies from <1% to >60%. There has even been a report of a rare quadruple malaria infection in a remote area in the western half of New Guinea Island (Irian Jaya Province, Indonesia); this infection was confirmed using nested PCR and species-specific primer pairs (19). In Africa, *P. ovale* occurs commonly in areas with Duffy-negative populations who are refractory to *P. vivax*, thus appearing to prevent the natural coexistence of the two species. However, the Duffy blood group-negative trait that protects many African populations from *P. vivax* infection is considered very rare or nonexistent in New Guinea. Since *P. ovale* is considered a very unusual finding outside of Africa, the natural occurrence of *P. ovale* and *P. vivax* together in the same patient is considered a very rare finding. It is also likely that this population could also become infected with the fifth human malaria, *P. knowlesi*.

Although antagonism and predominance of one *Plasmodium* species over the other in the human host have been described, differences in the methods and sensitivity of detection may have complicated the interpretation of results. It is generally accepted that *P. falciparum* predominates over *P. vivax* in mixed infections. *P. ovale* has been found in mixed infections and has been established in persons carrying *P. falciparum*, *P. vivax*, and *P. malariae* at the same time. Simultaneous patent infections with three or four *Plasmodium* species are not entirely rare findings in the blood of healthy carriers in regions of hyperendemic infection.

Interaction between *P. falciparum* and *P. vivax* is often reported to be suppressive. There are reciprocal seasonal changes in the prevalence of the two species that cannot be explained by changes in vector behavior, antimalarial drug use, or age. Changes may be due to competition for host cells, since *P. falciparum* causes dyserythropoiesis, leading to a decreased number of reticulocytes available for invasion by *P. vivax*. It is possible that some cross-species immunity may be involved. Also, patients with mixed *P. falciparum-P. vivax* infections are four times less likely to exhibit patent gametocytemia than were those with a *P. falciparum* infection alone. Thus, coinfection with *P. vivax* may attenuate infections with *P. falciparum*. Also, there appear to be no limitations to vector infections with multiple species of malaria, and transmission of more than one species to humans has been documented from a single mosquito.

Detection of mixed infections can be difficult due to different levels of parasitemia, low organism densities, and confusion among various morphologic criteria for identification to the species level; these problems have been well documented. Using PCR methods, it is likely that a higher detection rate of chronic and mixed malarial species will be possible (19).

Diagnosis

Although malaria is no longer endemic within the United States, it is considered to be life-threatening, and laboratory requests for blood smear examination and organism identification should be treated as STAT requests. Malaria is usually associated with patients having a history of travel within an area where malaria is endemic, although other routes of infection are well documented (Algorithm 26.1).

Frequently, for a number of different reasons, organism recovery and subsequent identification are more difficult than the textbooks imply. It is very important that this fact be recognized, particularly when one is dealing with a possibly fatal malaria infection.

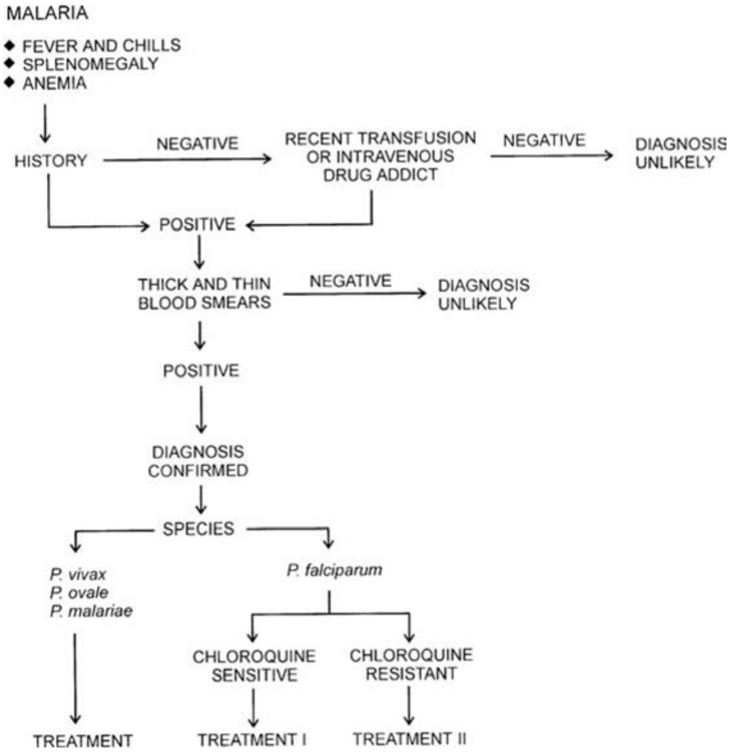
Patient Information. When requests for malarial smears are received in the laboratory, some patient history information should be made available to the laboratorian. This information should include the following.

1. Where has the patient been, and what was the date of return to the United States? (Also, "Where do you live?" has relevance to "airport" malaria.)
2. Has malaria ever been diagnosed in the patient before? If so, what species was identified?
3. What medication (prophylaxis or otherwise) has the patient received, and how often? When was the last dose taken?
4. Has the patient ever received a blood transfusion? Is there a possibility of other needle transmission (drug user)?
5. When was the blood specimen drawn, and was the patient symptomatic at the time? Is there any evidence of a fever periodicity?

Answers to such questions may help eliminate the possibility of infection with *P. falciparum* or *P. knowlesi*, usually the only species that can rapidly lead to death.

Conventional Microscopy. Often, when the diagnosis of malaria is considered, only a single blood specimen is submitted to the laboratory for examination; however, single films or specimens cannot be relied on to exclude the diagnosis, especially when partial prophylactic medication or therapy is used. Partial use of antimalarial agents may be responsible for reducing the numbers of organisms in the peripheral blood, thus leading to a blood smear that contains few organisms, which then implies a low parasitemia when in fact serious disease is present. Patients with a relapse case or an early primary case may also have few organisms in the blood smear (Table 26.6).

It is recommended that both thick and thin blood films be prepared on admission of the patient, and at least 200


MALARIA
◆ FEVER AND CHILLS
◆ SPLENOMEGALY
◆ ANEMIA
HISTORY
NEGATIVE
RECENT TRANSFUSION OR INTRAVENOUS DRUG ADDICT
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
THICK AND THIN BLOOD SMEARS
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
DIAGNOSIS CONFIRMED
SPECIES
P. vivax
P. ovale
P. malariae
P. falciparum
CHLOROQUINE SENSITIVE
CHLOROQUINE RESISTANT
TREATMENT
TREATMENT I
TREATMENT II

Algorithm 26.1 Malaria.

to 300 oil immersion fields should be examined on both films before a negative report is issued (20, 21). Since one set of negative films will not rule out malaria, additional blood specimens should be examined over a 36-h time frame. Although Giemsa stain is widely used for all parasitic blood work, the organisms can also be seen if other blood stains, such as Wright's stain, are used (Tables 26.7 and 26.8; Fig. 26.4 and 26.5). Blood collected with the use of EDTA anticoagulant is acceptable; however, if the blood remains in the tube for any length of time, true stippling may not be visible within the infected RBCs (*P. vivax*, as an example). Also, when using anticoagulants, it is important to remember that the proper ratio between blood and anticoagulant is necessary for good organism morphology. Heparin can also be used, but EDTA is preferred. Finger stick blood is recommended, particularly when the volume of blood required is minimal (i.e., when no other hematologic procedures have been ordered). The blood should be free flowing when taken for smear preparation and should not be contaminated with alcohol used to clean the finger prior to the stick. However, in areas of the world where automation for blood work is widely used, this method is less common than in the past.

Accurate species diagnosis is essential for good patient management, since identification to the species level may determine which drug or combination of drugs will be indicated. Some patients with *P. falciparum* infections may not yet have the crescent-shaped gametocytes in the blood. Low parasitemias with the delicate ring forms may be missed; consequently, oil immersion examination at ×1,000 is mandatory.

Parasite density generally correlates with disease severity, but peripheral parasitemia does not always reflect the number of sequestered organisms. Malaria pigment may serve as a peripheral indicator of parasite biomass, since the pigment can be seen within monocytes and polymorphonuclear leukocytes during light microscopy examination. The presence of pigment has been strongly associated with more severe disease than occurs with uncomplicated cases of malaria (Fig. 26.18). Pigmented neutrophils (polymorphonuclear leukocytes, monocytes) have been associated with cerebral malaria and with death in children with severe malaria (22).

Malaria is one of the few parasitic infections considered to be immediately life-threatening, and a patient with the diagnosis of P. falciparum *or* P. knowlesi *malaria should be considered a medical emergency because the disease can be rapidly fatal. Any laboratory providing the expertise to identify malarial parasites should do so on a 24-h basis, 7 days per week.*

Patients with malaria may appear for diagnostic blood work when least expected. Laboratory personnel should be aware of the STAT nature of such requests and the importance of obtaining some specific patient history information. The typical textbook presentation of the blood smears may not be seen by the technologist. It is very

TABLE 26.8 Plasmodia in Giemsa-stained thin blood films[a]

Characteristic	*Plasmodium vivax*	*Plasmodium malariae*	*Plasmodium falciparum*	*Plasmodium ovale*
Persistence of exoerythrocytic cycle	Yes	No	No	Yes
Relapses	Yes	No, but long-term recrudescences are recognized	No long-term relapses	Possible, but usually spontaneous recovery
Time of cycle (h)	44–48	72	36–48	48
Appearance of parasitized RBC (size and shape)	1.5 to 2 times larger than normal; oval to normal; may be normal size until ring fills half of cell	Normal shape; size may be normal or slightly smaller	Both normal	60% of cells larger than normal and oval; 20% have irregular, frayed edges
Schüffner's dots (eosinophilic stippling)	Usually present in all cells except early ring forms	None	None; comma-like red dots (Maurer's dots) occasionally present	Present in all stages including early ring forms; dots may be larger and darker than in *P. vivax*
Color of cytoplasm	Decolorized, pale	Normal	Normal, bluish tinge at times	Decolorized, pale
Multiple rings/cell	Occasional	Rare	Common	Occasional
All developmental stages present in peripheral blood	All stages present	Ring forms few, as ring stage is brief; mostly growing and mature trophozoites and schizonts	Young ring forms and no older stages; few gametocytes	All stages present
Appearance of young trophozoite (early ring form)	Ring 1/3 diameter of cell; cytoplasmic circle around vacuole; heavy chromatin dot	Ring often smaller than in *P. vivax*, occupying 1/8 of cell; heavy chromatin dot; vacuole at times "filled in"; pigment forms early	Delicate, small ring with small chromatin dot (frequently 2); scanty cytoplasm around small vacuoles; sometimes at edge of red cell (appliqué form) or filamentous slender form; may have multiple rings per cell	Ring larger and more ameboid than in *P. vivax;* otherwise similar to *P. vivax*
Appearance of growing trophozoite	Multishaped irregular ameboid parasite; streamers of cytoplasm close to large chromatin dot; vacuole retained until close to maturity; increasing amounts of brown pigment	Nonameboid rounded or band-shaped solid forms; chromatin may be hidden by coarse dark brown pigment	Heavy ring forms; fine pigment grains	Ring shape maintained until late in development; nonameboid compared with *P. vivax*
Appearance of mature trophozoite	Irregular ameboid mass; 1 or more small vacuoles retained until schizont stage; fills almost entire cell; fine brown pigment	Vacuoles disappear early; cytoplasm compact, oval, band shaped, or nearly round, almost filling cell; chromatin may be hidden by peripheral coarse dark brown pigment	Not seen in peripheral blood (except in severe infections); development of all phases following ring form occurs in capillaries of viscera	Compact; vacuoles disappear; pigment dark brown, less abundant than in *P. malariae*
Appearance of schizont (presegmenter)	Progressive chromatin division; cytoplastic bands containing clumps of brown pigment	Similar to *P. vivax* except smaller; darker, larger pigment granules peripheral or central	Not seen in peripheral blood (see above)	Smaller and more compact than *P. vivax*
Appearance of mature schizont	Merozoites, 16 (12–24), each with chromatin and cytoplasm, filling entire RBC, which can hardly be seen	8 (6–12) merozoites in rosettes or irregular clusters filling normal-size cells, which can hardly be seen; central arrangement of brown-green pigment	Not seen in peripheral blood (see above)	3/4 of cells occupied by 8 (8–12) merozoites in rosettes or irregular clusters

(continued)

TABLE 26.8 *(continued)*

Characteristic	*Plasmodium vivax*	*Plasmodium malariae*	*Plasmodium falciparum*	*Plasmodium ovale*
Appearance of macrogametocyte	Rounded or oval homogeneous cytoplasm; diffuse delicate light brown pigment throughout parasite; eccentric compact chromatin	Similar to *P. vivax*, but fewer; pigment darker and more coarse	Sex differentiation difficult; "crescent" or "sausage" shapes characteristic; may appear in "showers," black pigment near chromatin dot, which is often central	Smaller than *P. vivax*
Appearance of microgametocyte	Large pink to purple chromatin mass surrounded by pale or colorless halo; evenly distributed pigment	Similar to *P. vivax*, but fewer; pigment darker and more coarse	Same as macrogametocyte (described above)	Smaller than *P. vivax*
Main criteria	Large RBC; trophozoite irregular; pigment usually present; Schüffner's dots not always present; several phases of growth seen in one smear; gametocytes appear as early as day 3	RBCs normal in size and color; trophozoites compact, stain usually intense, band forms not always seen; coarse pigment; no stippling of RBCs; gametocytes appear after a few weeks	Development following ring stage takes place in blood vessels of internal organs; delicate ring forms and crescent-shaped gametocytes are the only forms normally seen in peripheral blood; gametocytes appear after 7–10 days	RBC enlarged, oval, with fimbriated edges; Schüffner's dots seen in all stages; gametocytes appear after 4 days or as late as 18 days

[a] Although *P. knowlesi* is not listed in this table, the overall morphology using Giemsa stain will be very similar to that seen for the other four species; see Fig. 26.5 and Table 26.2.

important that the smears be examined at length and under oil immersion. The most important thing to remember is that even though a low parasitemia may be present on the blood smears, the patient may still be faced with a serious, life-threatening disease. See Algorithm 26.1 for additional information.

Alternative Methods. Giemsa-stained thick and thin blood films have been used to diagnose malaria for many years and have always been considered the "gold standard." However, a number of alternative methods have been developed, including different approaches to microscopy, flow cytometry, biochemical methods, immunoassay, and molecular methods (Table 26.9). The aim of these procedures has been to reduce cost, reduce requirements for expensive equipment, increase sensitivity, and provide simple, rapid methods that do not require conventional microscopy (23–39).

Microhematocrit centrifugation. Microhematocrit centrifugation, using the QBC tube (glass capillary tube and closely fitting plastic insert [QBC malaria blood tubes; Becton Dickinson, Sparks, MD]), has been used for the detection of blood parasites. At the end of centrifugation of 50 to 110 μl of capillary or venous blood (5 min in a QBC centrifuge [14,387 × *g*]), parasites or RBCs containing parasites are concentrated into a small, 1- to 2-mm region near the top of the RBC column and are held close to the wall of the tube by the plastic float, thereby making them readily visible by microscopy. Tubes precoated with acridine orange provide a stain that induces fluorescence in the parasites. This method automatically prepares a concentrated smear that represents the distance between the float and the walls of the tube. The tube is placed into a plastic holder, a drop of oil is applied to the top of the hematocrit tube, a coverslip is added, and the tube is examined with a 40× to 60× oil immersion objective (the objective must have a working distance of 0.3 mm or greater).

Note Although a malaria infection can be detected by the microhematocrit centrifugation method (which is more sensitive than use of the thick or thin blood smear), *appropriate thick and thin blood films must be examined to accurately identify the species causing the infection.*

Fluorescent dye. Another approach uses the fluorescent dye benzothiocarboxypurine (Becton Dickinson, Mountain View, CA), which intensely stains nucleic acid. In fresh blood, the dye easily penetrates the RBC membrane and stains malarial parasites, with the asexual forms staining more intensely than the gametocytes. Under these circumstances, the WBC nuclei do not stain. However, when fixed, the dye penetrates and stains both malarial parasites and WBCs. The advantages of this alternative to Giemsa-

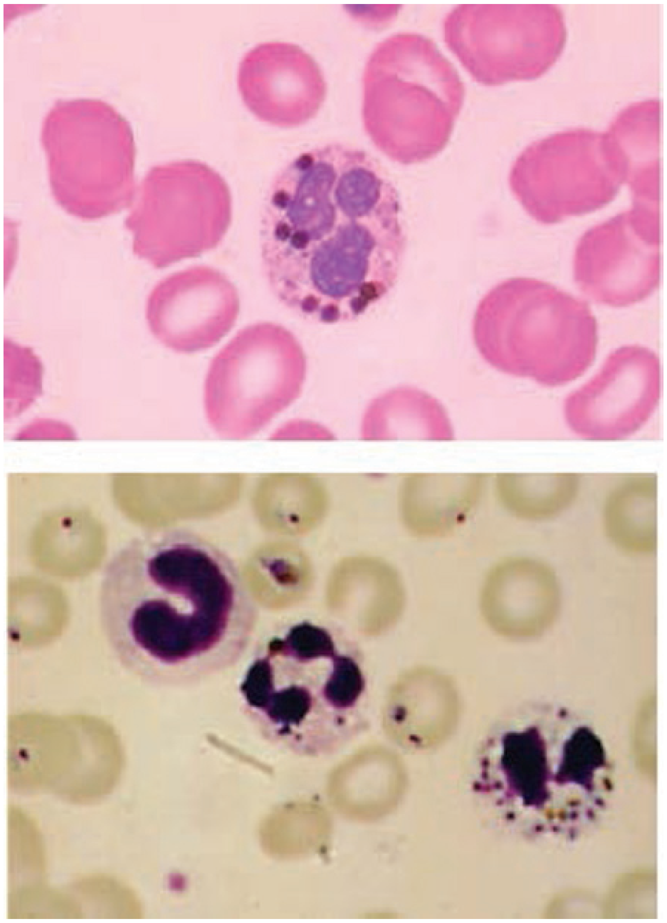


Figure 26.18 (Upper) Intracytoplasmic malaria pigment seen within a polymorphonuclear leukocyte in a specimen from a patient with malaria (courtesy of Loyda Oduber, Jaime LaRoche, Pitágoras Ureña, and Cinthia Batista, Clinical Hospital San Fernando, Panama). (Lower) Malaria pigment seen within a polymorphonuclear leukocyte (courtesy of George Healy, CDC). doi:10.1128/9781555819002.ch26.f18

stained blood films, particularly in field laboratories, are the speed of staining and evaluation and the relatively low level of training necessary to provide consistent results. Another fluorescent dye that is used is 4′,6-diamidine-2-phenylindolo-propidium iodide. However, the one disadvantage for field work is the requirement for a fluorescence microscope. Additional work is continuing on the use of benzothiocarboxypurine, and a stable prestained slide system that eliminates the need for liquid-stain application is now available.

Rapid diagnostic tests (RDTs). With the increase in drug-resistant *P. falciparum* cases in the tropics, it is no longer feasible to treat febrile patients empirically with inexpensive yet effective antimalarial agents. The substitution of newer, more expensive drugs mandates rapid, accurate, and inexpensive diagnostic procedures so that directed therapy can be provided. Immunochromatography relies on the migration of liquid across the surface of a nitrocellulose membrane. Using monoclonal antibodies prepared against a malaria antigen target that has been incorporated onto the strip of nitrocellulose, these tests are based on the capture of parasite antigen from peripheral blood. Currently, the malaria antigens used for these rapid diagnostic tests are histidine-rich protein 2 (HRP-2), parasite lactate dehydrogenase (pLDH), and *Plasmodium* aldolase (Table 26.9). These dipsticks offer the possibility of more-rapid, nonmicroscopic methods for malaria diagnosis.

A *P. falciparum* antigen detection system, the ParaSight-F test (Becton Dickinson Europe), has been very effective in field trials and has detected the HRP-2. This procedure is based on an antigen capture approach and has been incorporated in a dipstick format; the entire test takes approximately 10 min (Fig. 26.19). Another test that detects HRP-2, also in the dipstick format, is the ICT Malaria *Pf* (AMRAD ICT). This test appears to perform well and is comparable to the ParaSight-F test; however, the false-positive rate appears to be lower in this procedure. The third dipstick format procedure, OptiMAL (Flow Inc.), uses monoclonal antibodies against species-specific pLDH. Its sensitivity and specificity appear to be similar to those of the HRP-2 assays, with similar limitations at lower parasitemias. An advantage of this method is its use in follow-up for posttherapy patients to confirm a cure; the test detects only viable parasites. These rapid tests are easy to perform and interpret; however, there are a number of questions that remain concerning the relevant uses for this type of testing, especially considering the fact that the BinaxNow Malaria Test is FDA approved (Alere, Inc., Waltham, MA). A positive-control specimen is also available (Fig. 26.20).

Sensitivity remains a problem, particularly for nonimmune populations. Parasite densities of >100 parasites/μl (0.002% parasitemia) should be detected and are reasonable targets to expect from dipsticks for *P. falciparum* diagnosis. However, this level of sensitivity is at the lower end of the capability of most devices using capture methods for HRP-2 or pLDH (Table 26.6). This level of sensitivity is probably as good as clinical laboratory staff in nonspecialized laboratories with limited exposure to malaria cases could expect to provide using microscopy diagnosis. One of the potential benefits would be for inexperienced evening staff personnel for whom the dipstick identification of a life-threatening parasitemia with *P. falciparum* could prevent a missed infection. However, a negative test could not be accepted and would require confirmation by microscopic examination of both thick and thin blood films for the detection of parasitemia below the present threshold of detection by these rapid tests. Also, there are rare false

TABLE 26.9 Alternative approaches to malaria diagnosis[a]

Principle of the method	Method	Comments	Pros	Cons
Stained blood films, light microscopy[b]	TBF and THBF, after cytocentrifugation (100 µl of lysed blood centrifuged onto slide, stained with Giemsa)	Traditional method; commercially available (used in histopathology labs)	TBF used to screen larger amount of blood; THBF allows visualization of parasite within RBC; generally thought to be more sensitive than rapid antigen tests, particularly for ID to the species level	TBF unable to see parasite within RBC; THBF provides less blood per exam
Fluorescent DNA-RNA stains	TBF and THBF films (e.g., acridine orange)	Nonspecific	Sensitivity good	Requires expertise in reading
	After centrifugation (QBC)	Commercially available	Saves time, fairly sensitive	Expensive, species ID may be difficult
	Flow cytometry	Commercially available; low sensitivity	Automated counts	Low sensitivity due to "background noise"
Molecular methods	DNA-RNA hybridization	Poor sensitivity	"New approach"	Low sensitivity, cumbersome procedures
	PCR	Sensitive	Detect small numbers; species ID with mixed infections; used with blood spot collection	Lengthy procedure; automation may solve this problem
	Real-time PCR (QT-PCR)	Sensitive	Sensitivity of 20 parasites/ml of blood	Requires 500 µl of blood; takes 12 h longer than QT-NASBA
	Real-time nucleic acid sequence-based amplification (QT-NASBA)	Sensitive	Sensitivity of 20 parasites/ml of blood	Requires 50 µl of blood; takes 12 h less than QT-PCR; lends itself to testing large numbers of samples
Malaria pigment detection	Dark-field microscopy	Inferior to TBF	Pigment in WBCs sensitive indicator of prognosis	Not as sensitive as TBF
	Automated blood cell analyzers	Commercially available; detection of cases in patients not suspected as having malaria	Detects malaria pigment (WBCs, schizonts, gametocytes)	Nonspecific findings per species and parasitemia
	Depolarizing monocytes containing malarial pigment	Related to duration of symptoms before testing	Malarial pigment in monocytes	More obvious if parasitemia is ≥0.5%
	Pseudoreticulocytosis	Related to *P. falciparum*	Nuclear material from intraerythrocytic parasites	More obvious if parasitemia is ≥0.5%
Antigen detection (these tests were not developed with *P. knowlesi* in mind; other tests now under development)	HRP-2 assays: Paracheck Pf	Commercially available	Simple, rapid dipstick format; good correlation with conventional microscopy; may detect *P. falciparum* (chloroquine resistant) after patient treated for *P. vivax* (mixed infections)	*P. falciparum* only; drop in sensitivity at low levels of parasitemia (<100/µl); misses cases of only mature gametocytes (no HRP-2); false positives seen
	ICT Malaria *Pf*	Commercially available	Simple, rapid dipstick format; lower false-positive rate; good sensitivity, especially when coupled with microscopy	Some reagents must be stored at 2–8°C; cost may be a factor; more sensitive for placental malaria than microscopy

(continued)

TABLE 26.9 Alternative approaches to malaria diagnosis[a] *(continued)*

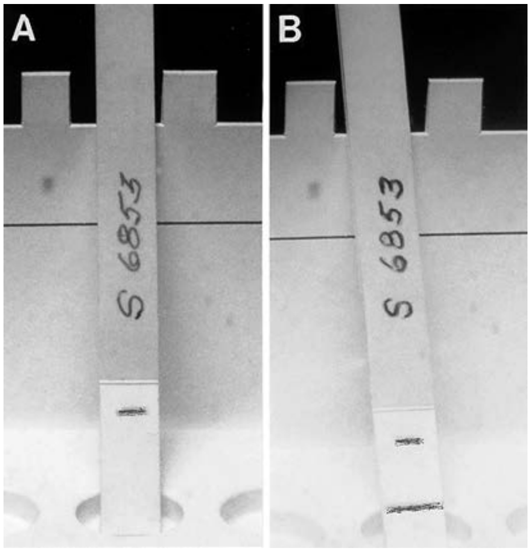
Principle of the method	Method	Comments	Pros	Cons
	MAKROmed	Commercially available	Antigen capture immunochromatographic strip format; more sensitive than conventional microscopy	Highly sensitive in detecting placental malaria, lower than expected specificity
	pLDH OptiMAL	Commercially available; test positive only with viable organisms	Simple, rapid dipstick format; also picks up *P. vivax*; few false positives; good test of cure (picks up viable parasites only)	Similar limitations at low parasitemias (<0.01%); mixed-infection ID difficult; cannot replace microscopy
	BinaxNOW (FDA-approved for use within the United States)	Commercially available; good sensitivity	Simple, rapid dipstick format; lower false-positive rate	Some reagents must be stored under refrigeration; cost may be a factor; insensitive for *P. ovale*, *P. malariae*, *P. knowlesi*

[a] Adapted from references 23–39 and 110; not all commercially available products are listed here. Very comprehensive lists of product testing to date may be found at World Health Organization (2012), http://apps.who.int/iris/bitstream/10665/77748/1/9789241504720_eng.pdf?ua=1 (accessed 4/8/2014). HRP-2, histidine-rich protein 2; ID, identification; pLDH, parasite lactate dehydrogenase; TBF, thick blood film; THBF, thin blood film; WBCs, white blood cells.

[b] Although for many years Giemsa stain has been the stain of choice, the parasites can also be seen on blood films stained with Wright's stain, a Wright/Giemsa combination stain, or one of the more rapid stains such as Diff-Quik (American Scientific Products, McGaw Park, IL), Wright's Dip Stat stain (Medical Chemical Corp., Torrance, CA), or Field's stain.

positives for patients with certain rheumatologic disorders; this is noted in the package insert and has been confirmed by users in this patient population. The U.S. military has conducted FDA-approved trials because of possible use of the devices in the field. It is very important to remember that the rapid tests currently available were not developed with the fifth human malaria, P. *knowlesi*, in mind; in some cases the tests have identified *P. knowlesi* as present, but not in all cases. Also cross-reactivity with other *Plasmodium* species has been noted. Specificity of pLDH-RDTs is decreased for *P. vivax* and *P. falciparum* due to *P. knowlesi* cross-reactivity, thus limiting their use alone in *knowlesi*-endemic areas. Sensitive *knowlesi*-specific RDTs and/or alternative molecular diagnostic tools are needed in *knowlesi*-endemic areas.

Figure 26.19 ParaSight-F test kit showing a negative test strip with a reagent control mark (A) and a positive test strip with a reagent control mark above the positive test result (B). doi:10.1128/9781555819002.ch26.f19



Overall, although they are rapid, simple, and easy to interpret, RDTs target proteins specific to *P. falciparum* or *P. vivax* or those common to all *Plasmodium* species and cannot specifically identify *P. malariae*, *P. ovale*, and *P. knowlesi*. Commercially available RDTs vary widely in diagnostic sensitivity (e.g., lacking sensitivity for some strains of *P. falciparum* and up to 50% of *P. knowlesi*) and specificity (e.g., *P. vivax* in patients with coinfections and high *P. falciparum* parasitemia levels). Lastly, RDTs cannot be used to monitor therapy, because they are qualitative. Availability of more than one test approved by FDA remains a problem.

Molecular tests. Nucleic acid amplification is the most sensitive and specific method to detect *P. falciparum*. However, PCR remains laboratory-based and requires trained personnel. Furthermore, the power dependency for the thermocycling process and the costly equipment necessary for the read-out are difficult to cover in resource-limited settings. PCR has proven to be one of the most specific

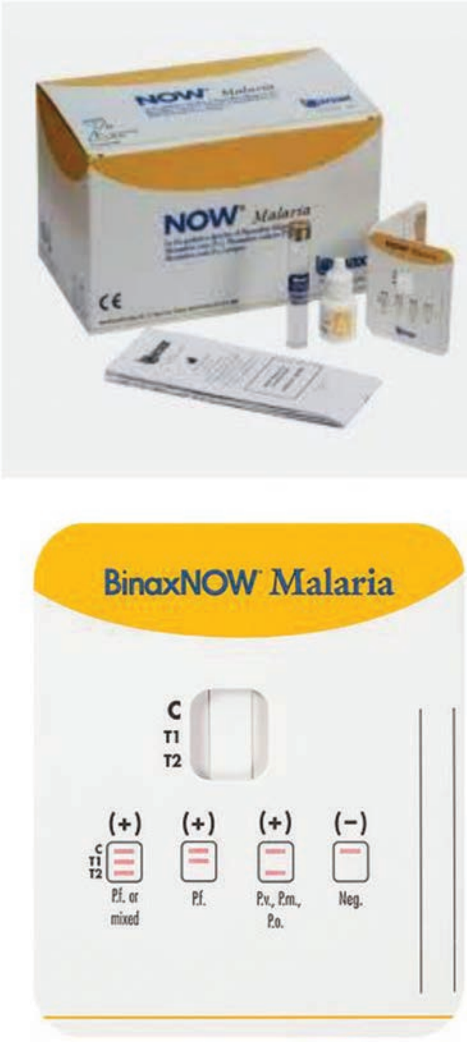


Figure 26.20 BinaxNOW Malaria test kit showing examples of possible answers. C, control line; T1, *Plasmodium falciparum* specific line; T2, panspecific line for all *Plasmodium* species (Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*; Pm, *Plasmodium malariae*; Po, *Plasmodium ovale*). **Note:** This test was not designed to detect the presence of *P. knowlesi*; the development of newer reagents for this species is under way. (Courtesy of Alere—FDA approved for use within the United States.) doi:10.1128/9781555819002.ch26.f20

and sensitive diagnostic methods, particularly for malaria cases with low parasitemia. However, this technique has limitations in its routine use under resource-limited conditions. A number of recent studies have reported on the benefits of using PCR for detection of malaria; the high sensitivity, rapidity, and simplicity of some of the methods should be applicable to large-scale epidemiology studies, follow-up of drug treatment, and immunization trials. Detection is possible with as few as 5 or 10 parasites per μl of blood, confirming that PCR detects many more cases of low-level parasitemia than do thick blood films (36, 40).

In one study, three quantitative real-time PCR methods (qRT-PCR) were tested: qRT-PCR1 amplified a specific sequence on the *P. falciparum* Cox1 gene, qRT-PCR2 amplified a species-specific region of the multicopy 18S rDNA, and qRT-PCR3 amplified a mitochondrial DNA sequence. These three rapid, molecular qRT-PCR methods, used alone or in association, showed excellent results, with high concordance, accuracy, and reliability in malaria diagnosis (41).

In another study, the isothermal recombinase polymerase amplification method was combined with lateral flow detection as an approach to improve molecular diagnostics for *P. falciparum* in resource-limited settings. The system requires little to no instrumentation for the nucleic acid amplification reaction and the read-out is possible with the naked eye. Showing the same sensitivity and specificity as comparable diagnostic methods but simultaneously increasing reaction speed and dramatically reducing assay requirements, the method has potential to become a true point-of-care test for the malaria parasite (42).

A high-throughput multiplex 5′ nuclease quantitative PCR (qPCR) assay has been developed, with the potential to support large studies, to specifically identify *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. The analytical limit of sensitivity for each *Plasmodium* species component of the 5-plex assay was 10 to 100 copies/μl, similar to that of the 4-plex qPCR, and detection was linear from 10^1 to 10^5 copies. Based on the five-part multiplex PCR compared with microscopy, sensitivities ranged from 95.8 to 100% and specificities ranged from 98.6 to 100% (43).

In a recent study using a new loop-mediated amplification (LAMP) kit (Malaria LAMP, Eiken Chemical, Japan) in febrile returned travelers, this approach obtained diagnostic accuracy similar to that of nested PCR, and superior to that of expert microscopy. This current format of pan-*Plasmodium* genus and *P. falciparum*-specific LAMP is able to identify all *P. falciparum*-infected individuals. Nonfalciparum *Plasmodium* species are all identified but not resolved to the species level, a limitation shared with most RDT tests (44). In a companion study, the same Malaria LAMP kit was evaluated for samples from 272 outpatients at a rural Ugandan clinic and compared with expert microscopy, nested PCR, and qPCR. Compared with 3-well nested PCR, the sensitivity of both LAMP and single-well nested PCR was 90%; the microscopy sensitivity was 51%. For samples with a *P. falciparum* qPCR titer of ≥2 parasites/μl, LAMP sensitivity was 97.8% (95% confidence interval, 93.7–99.5%). Most false-negative LAMP results involved samples with parasitemia levels detectable by 3-well nested PCR but very low or undetectable by qPCR

(45). The current format has the potential to replace microscopy in developed country settings and to decrease the time lag prior to diagnosis in returning travelers.

Automated differential instruments. Hematology analyzers evaluated for malaria detection include the Cell-Dyn (Abbott Diagnostics, Santa Clara, CA), the GEN.S and LH-750 (Beckman Coulter, Miami, FL), and the XE-2100 and XS-1000*i* (Sysmex Corporation, Kobe, Kansai, Japan). The Sysmex XE-2100 is an automated hematology analyzer that uses flow cytometry, direct current impedanciometry, and radiofrequency conductance to detect and measure blood corpuscular elements. Flow cytometry may detect malaria parasites or parasite debris such as free hemozoin or hemozoin-laden macrophages as reported for Cell-Dyn instruments. For the Sysmex XE-2100, there is a gap between the manual and automated eosinophil counts (pseudoeosinophilia) and abnormal granulocyte-coded events in the DIFF scatterplot used for WBC separation based on fluorescence versus side-scatter signals in samples from *Plasmodium vivax*-infected patients. Two previously reported abnormalities, compared with thick film, had a sensitivity of 69.4% and a specificity of 100% for *P. vivax* diagnosis. These findings suggest the potential clinical utility of malaria detection by the XE-2100. However, the lack of scatterplot abnormalities in the *P. falciparum* samples, containing mainly rings, further supports the hypothesis that most abnormalities detected by the XE-2100 and other analyzers correspond to mature parasites, as found in *P. vivax* malaria. Again, the low parasitemias found in travelers (immunologically naive patients) definitely limit the potential for diagnosis using automated hematology instruments. The most common WBC and platelet flags tend to be leukopenia, atypical lymphocytes, lymphopenia, WBC abnormal scattergram, platelet clumps, thrombocytopenia, and platelet abnormal distribution. These instruments may provide valuable diagnostic parameters in areas of endemicity in detecting acute *P. vivax* malaria; however, they are not very useful for acute *P. falciparum* malaria infection.

Potential diagnostic problems with the use of automated differential instruments have been reported (37, 46). Some cases of malaria, as well as *Babesia* infection, have been completely missed by these methods. The number of fields scanned by a technologist on instrument-read smears is quite small; thus, failure to detect a light parasitemia is almost guaranteed. With both malaria and babesiosis, after diagnosis had been made on the basis of smears submitted to the parasitology division of the laboratory, all previous smears examined by the automated system were reviewed and found to be positive for parasites. Failure to make the diagnosis resulted in delayed therapy. Although these instruments are not designed to detect intracellular blood parasites, the inability of the automated systems to discriminate between uninfected RBCs and those infected with parasites may pose serious diagnostic problems in situations where the parasitemia is ≥0.5%.

Digital review of blood films. Preliminary studies are under way to develop semiautomated or automated methods for the detection and identification of *Plasmodium* species from digital stained blood film images. However, as with the automated hematology analyzers, low parasitemias may present problems with false-negative results. This may be a potential problem with travelers who present with low parasitemias, but who may be seriously ill.

Artifacts seen on blood films prepared from EDTA (time lag problems). Microscopic examination of a peripheral blood smear occasionally reveals ookinetes of *P. vivax* (or another of the *Plasmodium* species); this represents a potential "artifact" that could lead to the incorrect diagnosis of *P. falciparum* infection (Fig. 26.13). The ookinetes tend to resemble the crescent-shaped gametocytes seen in infections with *P. falciparum*. The appearance of these stages in the peripheral blood, rather than the mosquito gut, was probably due to the delay between blood collection and smear preparation. It has been documented that some gametocytes (*P. falciparum*) exflagellate at room temperature (pH 7.4). In this case, the microgametes could resemble spirochetes (8) (Fig. 26.6). These potential artifact problems usually arise when the tube of EDTA blood containing parasites is left standing at room temperature with the stopper removed. As the blood cools and becomes aerated, the *Plasmodium* parasites transition from the erythrocytic cycle in the human host to the cycle within the mosquito host. There may be other changes, including loss of parasites after 4 to 6 hours, changes in parasite shapes, changes in or loss of stippling, and overall morphology distortions. Therefore, it is important to prepare thick and thin blood films immediately after receipt of the blood (ordered, collected, processed, examined, and reported on a STAT basis) (21).

KEY POINTS—LABORATORY DIAGNOSIS

Malaria

1. Blood films should be prepared on admission of the patient (ordering, collection, processing, examination, reporting on a STAT basis). A fever pattern may not be apparent early in the course of the infection (immunologically naive patient, travelers); symptoms may be completely random and may mimic any other condition with vague complaints.
2. Both thick and thin blood films should be prepared. At least 200 to 300 oil immersion fields (×1,000) onboth thick and thin films should be examined before the specimen is considered negative.

KEY POINTS—LABORATORY DIAGNOSIS

3. Wright's, Wright-Giemsa, Giemsa, or a rapid stain can be used. The majority of the original organism descriptions were based on Giemsa stain. However, if the WBCs appear to be well stained, any blood parasites present will also be well stained. The WBCs on the patient smear serve as the quality control organism; there is no need to use a *Plasmodium*-positive slide for quality control.
4. Malarial parasites may be missed with the use of automated differential instruments. Even with technologist review of the smears, a light parasitemia is very likely to be missed.
5. The number of oil immersion fields examined may have to be increased if the patient has had any prophylactic medication during the past 48 h (the number of infected cells may be decreased on the blood films).
6. *One negative set of blood smears does not rule out malaria. Quantitate organisms from every positive blood specimen.* The same method for calculating parasitemia should be used for each subsequent positive blood specimen. Always report stages seen (rings, schizonts, gametocytes), since this information will influence therapy.
7. In spite of new technology, serial thick-film parasite counts are a simple, cheap, rapid, and reliable method for identifying patients at high risk of recrudescence due to drug resistance and treatment failure.
8. If you are using any of the alternative methods, make sure you thoroughly understand the pros and cons of each compared with the thick and thin blood film methods; see chapter 7 for additional information on diagnostic methods for blood parasites.

Report Comments

Report comments can be extremely helpful in conveying information to the physician. Depending on the results of diagnostic testing, the following information can lead to improved patient care and clinical outcomes. The report is provided with the comment following.

1. **No parasites seen:** The submission of a single blood specimen will not rule out malaria; submit additional bloods every 4 to 6 hours for 3 days if malaria remains a consideration.

Interpretation/Discussion: It is important to make sure the physician knows that examination of a single blood specimen will not rule out malaria.

2. ***Plasmodium* spp. seen:** Unable to rule out *Plasmodium falciparum* or *Plasmodium knowlesi.*

Interpretation/Discussion: Since *P. falciparum* and *P. knowlesi* cause the most serious illness, it is important to let the physician know these species have NOT been ruled out.

3. ***Plasmodium* spp., possible mixed infection:** Unable to rule out *Plasmodium falciparum* or *Plasmodium knowlesi.*

Interpretation/Discussion: Since *P. falciparum* and *P. knowlesi* cause the most serious illness, it is important to let the physician know these species have NOT been ruled out.

4. ***Plasmodium malariae*:** Unable to rule out *Plasmodium knowlesi.*

Interpretation/Discussion: If the patient has traveled to an area where *P. knowlesi* is endemic, it may be impossible to differentiate between *P. malariae* (band forms) and *P. knowlesi.*

5. ***Plasmodium falciparum*:** Unable to rule out *Plasmodium knowlesi.*

Interpretation/Discussion: If the patient has traveled to an area where *P. knowlesi* is endemic, it may be impossible to differentiate between *P. falciparum* (ring forms) and *P. knowlesi.*

6. **Negative for parasites using automated hematology analyzer:** Automated hematology analyzers will not detect low malaria parasitemias seen in immunologically naive patients (travelers).

Interpretation/Discussion: In patients who have never been exposed to malaria (immunologically naive), they will become symptomatic with very low parasitemias that will not be detected using automation (0.001 to 0.0001%).

7. **Negative for malaria using the BinaxNOW rapid test:** This result does not rule out the possibility of a malaria infection. Blood should be submitted for STAT thick and thin blood film preparation, examination, and reporting.

Interpretation/Discussion: The maximum sensitivity of this rapid test occurs at 0.1% parasitemia. Patients (immunologically naive travelers) may present to the emergency room or clinic with a parasitemia much lower than 0.1%, leading to a false-negative report. Also, this rapid test is not designed to identify P. malariae, P. ovale, *and* P. knowlesi*; the results are most clinically relevant for* P. falciparum *and* P. vivax. *The BinaxNOW is FDA approved for use within the United States.*

Treatment

Malaria has become a more common health problem during the last few years, both in residents of areas where the disease is endemic and in travelers returning to areas where

TABLE 26.10 Antimalarial drugs and their actions[a,b]

Stage affected	Antimalarial action by interference with: Folic acid synthesis	Folic acid metabolism	Nucleic acid metabolism	Iron-dependent free-radical generation	Parasite components	Unknown
Erythrocytic cycle (schizonts)	Sulfonamides Sulfones	Proguanil Pyrimethamine	Quinine[c] Amodiaquine[e]	Artemisinins	Chloroquine[d] (food vacuole function) Atovaquone (acts on mictochondria)	
Erythrocytic cycle (gametocytes)		Proguanil	Proguanil			Primaquine[f]
Exoerythrocytic cycle		Pyrimethamine	Pyrimethamine			Primaquine

[a] Format adapted from Markell EK, Voge M, *Medical Parasitology*, 5th ed., The WB Saunders Co, Philadelphia, PA, 1981; information updated from references 49, 111, and 112.

[b] Not every drug is included in this table; however, additional information can be seen in the references mentioned in footnote *a*.

[c] Affects the formation of malarial pigment; may also block ion transport to the parasite; inhibits production of tumor necrosis factor.

[d] Interferes with the normal function of the food vacuole and hence with maturation through the trophozoite stage; specific mechanisms include (i) binding to free heme to prevent its incorporation into malaria pigment, (ii) inhibiting aspartic and cysteine protease activity, and (iii) raising vacuolar pH above the pH optima of the aspartic and cysteine proteases in the food vacuole. Inhibition of DNA synthesis is no longer considered a likely mechanism for chloroqine action against the parasite.

[e] Amodiaquine binds heme and inhibits heme polymerization; this is an action similar to that seen with chloroquine.

[f] Primaquine is effective against gametocytes of all five species, unlike some of the other drugs which are partially effective; following administration of primaquine, documented swelling of mitochondria in malarial parasites.

it is not endemic. Therapy has become more complex because of the increase in resistance of *P. falciparum* to a variety of drugs and because of advances in treatment of severe disease complications (Tables 26.10 to 26.12). Antimalarial drugs are classified by the stage of malaria against which they are effective. These drugs are often referred to as tissue schizonticides (which kill tissue schizonts), blood schizonticides (which kill blood schizonts), gametocytocides (which kill gametocytes), and sporonticides (which prevent formation of sporozoites within the mosquito). To ensure that proper therapy is given, it is important for the clinician to know what species of *Plasmodium* is involved, the estimated parasitemia, and the geographic and travel history of the patient to determine the area where infection was probably acquired and the possibility of drug resistance related to that geographic area. The use of oral or parenteral therapy will be determined by the clinical status of the patient. A number of studies related to the molecular genetics of drug resistance, particularly in *P. falciparum* malaria and modeling of the development of resistance of *P. falciparum* to antimalarial drugs, are ongoing (Tables 26.13 to 26.15).

Development of resistance to the inexpensive antimalarials, such as chloroquine, has had a tremendous financial and public health impact on developing countries. In the late 1980s, chloroquine-resistant *P. falciparum* completely covered Africa.

Although chloroquine-resistant *P. falciparum* is well recognized, chloroquine has generally been effective for both chemoprophylaxis and treatment of *P. vivax*, *P. ovale*, and *P. malariae* infections. Cases of chloroquine-resistant *P. vivax* (CRPV) malaria have now been identified in Papua New Guinea, Indonesia, Malaysia, Vietnam, and Myanmar. CRPV is now endemic in Malaysia, Myanmar, and Vietnam. However, there is no evidence of CRPV from Thailand, Cambodia, Laos, China, the Korean Peninsula, or the Philippine archipelago (9). Although CRPV has been documented in a few areas within South America (Guyana, Colombia, Brazil, Peru), it is present at a very low frequency, with a risk of infection at <5%. Population-based epidemiologic studies are necessary to define the geographic range and prevalence of CRPV. It is not clear whether this development represents a newly emerging public health problem; however, clinicians need to be aware that possible therapeutic or prophylactic failures may result when chloroquine is used for *P. vivax* infections in people who have been in Indonesia or Oceania.

Suppressive Prophylaxis Therapy. Chemoprophylactic agents to prevent clinical symptoms are given to individuals who are going into areas where malaria is endemic (47). These drugs are effective only against the erythrocytic forms and do not prevent the person from getting malaria; i.e., they do not prevent sporozoites from entering the liver and beginning the pre-erythrocytic cycle of development. The most commonly used drugs for this purpose are quinine, chloroquine, hydroxychloroquine, and amodiaquine, all of which are effective when the organisms enter the RBCs from the liver and begin the erythrocytic cycle. Fansidar is recommended for prophylaxis only for travelers who are staying for a long time in high-transmission areas where choroquine-resistant malaria is endemic. This recommendation was based on revisions in response to increased

TABLE 26.11 *Plasmodium falciparum* drugs and therapeutic use[a]

Drug	Mechanisms of resistance	Clinical pharmacokinetics	Therapeutic use	Adverse effects and drug interactions
4-Aminoquinolones				
Chloroquine	Reduced cellular accumulation of drug; drug efflux mechanism; *pfcrt* major chloroquine resistance gene; resistance widespread	Rapidly absorbed after oral, intramuscular, or subcutaneous administration; half cleared by kidneys; terminal elimination half-life, 1–2 mo	*P. vivax*, *P. ovale*, *P. malariae*, *P.knowlesi*; uncomplicated *P. falciparum* in semi-immune patients; nonimmune patients require other drugs due to risks of drug failure	Well tolerated; >250 µg/ml causes dizziness, headache, diplopia, nausea, and exacerbation of epilepsy; cumulative dose of >100 g over time leads to irreversible retinopathy
Antifolate drugs[b] and combinations	Gene amplification of the first enzyme in the parasite folate synthesis pathway, GTP-cyclohydrolase, is strongly associated with resistant parasites and potentially contributes to persistence of resistant parasites.	Pharmacokinetic factors contribute to the observed high rate of treatment failure; a higher sulfadoxine-pyrimethamine dose for children under the age of 5 years is recommended; pharmacokinetics may be altered in pregnancy due to increases in oral clearance and volume of distribution	Median plasma concentrations are higher in nonpregnant versus pregnant subjects at most time points; higher doses can be given safely; differences due to physiological changes that are established in the first trimester	Severe allergic reactions to sulfa drugs well known; dapsone has concentration-related problems (allergy, problems with G6PD deficiency); pyrimethamine, proguanil, chlorproguanil have little serious toxicity other than folate deficiency
Sulfadoxine-pyrimethamine	Both long elimination half-lives, causing selective pressure for resistance; mutations in *dhfr* and *dhps* genes	Pyrimethamine well absorbed after oral or intramuscular administration; sulfa drugs given for synergistic effects with pyrimethamine or chlorproguanil	Used to treat chloroquine-resistant infections in Africa; severe skin reactions mean that it has no prophylaxis use	Skin reactions, anemia, leukopenia; single-dose therapy, but widespread resistance
Proguanil	Prophylaxis, but deteriorating clinical response; short half-life; atovaquone-proguanil-resistant *P. falciparum* rare	Atovaquone inhibits parasite mitochondrial electron transport. Proguanil inhibits the dihydrofolate reductase step in purine synthesis and lowers concentration of atovaquone necessary to kill *Plasmodium* spp.	Alone for prophylaxis; fixed-ratio combination with atovaquone (Malarone) (see below)	Nausea, vomiting, abdominal pain, hepatitis
Chlorproguanil[c] and dapsone	Converted to the active metabolite by cytochrome P450 enzymes; these metabolites inhibit the malaria parasite's dihydrofolate reductase enzyme.	Used for treatment; short half-life; low propensity to select resistant parasites	Treatment of uncomplicated *P. falciparum*; can be used during pregnancy	Associated risk of hemolytic anemia in G6PD-deficient patients; dapsone known to reduce erythrocyte lifespan
Quinine	Less potent than chloroquine, small therapeutic range; resistance rare in Africa; not major clinical problem	Oral bioavailability high; intramuscular half-life varies at 10 to 40 min; extensive hepatic biotransformation	Used in combination with antibiotics (tetracycline) in Southeast Asia; parenteral first choice for severe malaria	Tinnitus, deafness, headache, nausea, visual disturbances in the majority of patients receiving therapeutic levels; however, dose reduction not recommended; more serious sequelae can be seen

(continued)

TABLE 26.11 *Plasmodium falciparum* drugs and therapeutic use[a] *(continued)*

Drug	Mechanisms of resistance	Clinical pharmacokinetics	Therapeutic use	Adverse effects and drug interactions
Quinidine gluconate	Narrow therapeutic window; kills parasites in late stages of RBC cycle	Same mechanisms as quinine	Treatment of severe malaria	Infusion-related hypotension, QT prolongation; potential cardiotoxicity; can induce hypoglycemia (promotes insulin secretion)
Artemisinin group	Artemether, artesunate, dihydroartemisinin semisynthetic derivatives of plant extract; high-cost drugs; decreased effectiveness seen along border between Cambodia and Thailand (use of monotherapy)	Rapidly hydrolyzed *in vivo* to dihydroartemisinin; short elimination half-lives; artemether absorption slower, more variable if administered intramuscularly or intravenously	Used to treat severe malaria and as combination therapy	Generally safe and well tolerated; main concern is reproductive safety (long-bone shortening); cannot be used in first trimester (WHO)
Artemether-lumefantrine[d]	Resistance not yet detected; monitoring ongoing (*pfmdr1*-86, *pfcrt*-76, *pfmdr1*-184, 1042, 1246, *pfmdr1* gene copy number)	Take with fatty foods to increase absorption	Lumefantrine, a fluorene derivative, may interfere with heme metabolism. Artemether-lumefantrine is rapidly effective against all RBC stages.	Nausea, vomiting, dizziness, headache, possible QT prolongation

[a] Adapted from references 111–114. FDA-approved drugs include chloroquine, doxycycline (prophylaxis), mefloquine, primaquine, pyrimethamine, quinidine gluconate, quinine Coartem (artemether-lumefantrine), Fansidar (pyrimethamine-sulfadoxine), Malarone (atovaquone-proguanil). The QT interval is a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. The QT interval represents electrical depolarization and repolarization of the ventricles. A lengthened QT interval is a marker for the potential of ventricular tachyarrhythmias and a risk factor for sudden death.

[b] **Heinberg A, Kirkman L,** *Ann NY Acad Sci* **1342:**10-18, 2015.

[c] **Tiono AB et al,** *Am J Trop Med Hyg* **81:**969-978, 2009.

[d] **Shah M et al,** *Malaria J* **14:**75-89, 2015.

TABLE 26.12 Antimalarial drugs and associated problems[a]

Drug	Associated problems[b]
Artemisinin (qinghaosu)	Recrudescence, neurotoxity; increased convulsions; short half-life
Atovaquone (Mepron)	Rash, nausea; rapid development of resistance when used alone; recrudescence common
Azithromycin	Limited use; efficacy to be determined
Chloroquine (Aralen)	Resistance worldwide except in Central America; appears to increase *P. falciparum* gametocytogenesis
Doxycycline	Phototoxicity; not used in pregnant women and children; gastrointestinal intolerance
Halofantrine (Halfan)	Cardiotoxicity, poor absorption, sporadic resistance
Mefloquine (Lariam)	Psychoses; resistance in Indochina and Africa
Primaquine	Narrow therapeutic index; not used in G6PD-deficient patients; resistance may be a problem
Proguanil (Paludrine)	Mouth ulcers, hair loss, scaling of palms and soles, urticaria; resistance worldwide
Pyrimethamine-sulfadoxine (Fansidar)	Severe allergic reactions; resistance worldwide
Quinine sulfate	Tinnitus, headache, nausea, abdominal pain, visual disturbances; resistance in Brazil and Indochina
Quinidine gluconate	Infusion-related hypotension, hypoglycemia; requires cardiac monitoring

[a] Adapted from references 115–117.

[b] G6PD, glucose-6-phosphate dehydrogenase.

TABLE 26.13 Malaria resistance[a]

Resistance definition	Comments
Sensitive	From initiation of therapy, asexual parasites are cleared by day 6; no evidence of recrudescence up to day 28 Peripheral blood films appear to go from positive to negative very quickly (can be a change from one draw to the second draw 6 h later)
Resistance type I (RI)	From initiation of therapy, asexual parasites have cleared for at least 2 consecutive days (the latest day being day 6); recrudescence follows Parasite count initially drops and blood films appear to be negative; patient should be monitored for a period of days, particularly if drug-resistant *P. falciparum* suspected
Resistance type II (RII)	Within 48 h of initiation of therapy, marked reduction of asexual parasitemia to <25% of pretreatment count; however, no subsequent disappearance of parasitemia (smear positive on day 6) Patient appears to be improving; parasite count drops, but blood films always appear positive
Resistance type III (RIII)	Modest reduction in parasitemia may be seen; no change or increase in parasitemia seen during first 48 h after treatment; no clearing of asexual parasites In some cases, parasite count continues to increase with no visible decrease at any time; blood films show overall parasite increase

[a] Adapted from references 107 and 116.

numbers of adverse reactions to Fansidar prophylaxis, including severe mucocutaneous reactions, some of which were fatal (Table 26.12). In general, chloroquine is still the choice for prophylaxis against *P. falciparum*, but it is not totally effective against *P. vivax*. Resistance to Fansidar was reported in Thailand as early as 1981. Quinine is normally used in these situations; however, quinine-resistant *P. falciparum* has been reported in Africa, with little to no information on previous chloroquine intake. A combination of quinine and tetracycline is recommended for chloroquine- and Fansidar-resistant *P. falciparum*. Areas with chloroquine-resistant *P. falciparum* are listed in Table 26.14, and drug information is presented in Tables 7.15 and 7.16; specific dosage information is given in chapter 36.

Summary of Prophylaxis. With the introduction of chloroquine, with its high potency, long half-life, and low toxicity, continued research into the chemoprophylactic potential of other antimalarial agents such as primaquine was drastically reduced. Resistance to the newer chemoprophylactic drugs such as chloroquine, sulfadoxine/pyrimethamine (Fansidar), proguanil, mefloquine, and doxycycline continues to increase, with many of these agents now becoming clinically useless. Other antimalarial agents such as quinine, halofantrine, or the artimisinine derivatives are too toxic or have too short a half-life to be used for prophylaxis. A summary of antimalarial drugs used for prophylaxis and their activity on different life cycle stages of the malaria parasite can be seen in Table 26.10.

Perhaps the most common approach to chemoprophylaxis in travelers to areas with multidrug-resistant *P. falciparum* is the use of doxycycline. However, this tetracycline derivative must be taken daily, and its use must be continued for 4 weeks after the patient has left the area. Potential side effects include diarrhea, upper gastrointestinal upset, and phototoxicity. Patients may also be predisposed to oral or vaginal candidiasis, and the drug cannot be given during pregnancy or to young children. Also, breakthrough can occur in individuals who have missed only a single dose.

Every year between 10,000 and 30,000 international travelers from nonendemic areas fall ill with malaria after returning home. Estimates indicate that around 150 returning travelers die each year from imported malaria, usually due to *P. falciparum* infection. In areas of intense malaria transmission, chemoprophylaxis remains the most important strategy for preventing malaria in travelers, but its use may be associated with adverse outcomes and even death. These potentially severe side effects often undermine compliance with chemoprophylaxis in travelers. Another problem with malaria chemoprophylaxis is that all drug regimens must be taken meticulously during and for 1 or more weeks after leaving the malaria-endemic area, a commitment that requires considerable personal discipline.

Radical Cure. The radical-cure approach to therapy eradicates all malarial organisms, both the liver and the RBC stages, from the body. Therapy is usually given to individu-

TABLE 26.14 Areas with reported chloroquine-resistant *Plasmodium falciparum*[a]

- **Africa**
  - Angola
  - Benin
  - Botswana (Nov–June, northern areas)
  - Burkina Faso
  - Burundi
  - Cameroon
  - Central African Republic
  - Chad
  - Comoros
  - Congo
  - Côte d'Ivoire
  - Djibouti
  - Equatorial Guinea
  - Ethiopia (some areas)
  - Gabon
  - Gambia
  - Ghana
  - Guinea
  - Guinea-Bissau
  - Kenya
  - Liberia
  - Madagascar
  - Malawi
  - Mali
  - Mauritania (some areas)
  - Mozambique
  - Namibia (Nov–June, northern rural areas)
  - Niger
  - Nigeria
  - Rwanda
  - São Tomé and Principe
  - Senegal
  - Sierra Leone
  - Somalia
  - South Africa (northern and eastern Transvaal)
  - Sudan
  - Swaziland (northern and eastern plains)
  - Togo
  - Uganda
  - United Republic of Tanzania (some areas)
  - Zaire
  - Zambia (all year in Zambezi valley, Nov–June rest of country below1,200 m)
  - Zimbabwe
- **Asia**
  - Afghanistan (May–Nov)
  - Bhutan (southern areas)
  - Cambodia[b]
  - China (Hainan Island and southern provinces)
  - Indonesia[c]
  - Iran (Mar–Nov)
  - Lao People's Democratic Republic[d]
  - Malaysia
  - Myanmar
  - Nepal (areas other than Kathmandu, northern high-altitude areas)
  - Oman
  - Philippines (Luzon, Basilan, Mindoro Palawan, and Mindanao islands; Sulu Archipelago)
  - Saudi Arabia (some areas)
  - Sri Lanka (some areas)
  - Thailand
  - Vietnam
  - Yemen (Sept–Feb, some areas)
- **Oceania**
  - Papua New Guinea
  - Solomon Islands
  - Vanuatu
- **Indian subcontinent**[b]
  - Bangladesh (north and east)
  - India
  - Pakistan (Rawalpindi)
- **South America**
  - Bolivia
  - Brazil[e]
  - Colombia
  - Ecuador[f]
  - French Guiana
  - Guyana
  - Panama (east of the Canal Zone including the San Blas Islands)
  - Peru (northern provinces)
  - Suriname (some areas)
  - Venezuela (some areas)

[a] There is no malaria risk in urban areas unless otherwise indicated. This table should be used in conjunction with the text in determining appropriate prophylaxis. (From *MMWR Morb Mortal Wkly Rep* **34**[14], 1985; also see reference 118.)

[b] Malaria risk exists in most urban areas.

[c] Malaria risk exists in urban areas of Timor and Kalimantan provinces. Irian Jaya should be considered as Oceania.

[d] Malaria risk exists in all urban areas except Vientiane.

[e] Malaria risk exists in urban areas of interior Amazon River region.

[f] Malaria risk exists in urban areas of Esmeralda, Manabi, El Oro, and Guayas provinces (including the city of Guayaquil). (From Seidel JS [ed], *Pediatr Clin North Am:* **32:**1077–1096, 1985)

als who have returned from areas where malaria is endemic; it prevents relapses with *P. vivax* or *P. ovale* infection, although relapses with both *P. vivax* and *P. ovale* infections occasionally occur after treatment with primaquine (8, 48). The gametocytes are also eliminated, thus stopping the chain of transmission to the mosquito vector. The drugs used are primaquine and other 8-aminoquinolones. Treatment with primaquine is usually not necessary for malarial cases acquired by transfusion or contaminated needles or passed from mother to child as a congenital infection. More specific information can be found in chapter 36.

TABLE 26.15 Drugs available in the United States for treatment of malaria[a]

Tablets for oral use	
Atovaquone-proguanil (Malarone)	250 mg of atovaquone/100 mg of proguanil
Chloroquine phosphate (Aralen and generics)	500 mg (300-mg base), 1,000 mg (600-mg base)
Hydroxychloroquine sulfate (Plaquenil)	400 mg (310-mg base)
Mefloquine (Lariam and generics)	500 mg (456-mg base), 750 mg (684-mg base)
Primaquine phosphate	52.6 mg (30-mg base)
Pyrimethamine (Daraprim)	25 mg
Quinine	180 mg, 325 mg
Quinine sulfate	650 mg (542-mg base)
Ampoules for parenteral use	
Chloroquine hydrochloride (200-mg chloroquine base, 5-ml vial) and quinine dihydrochloride	
Quinidine gluconate, 800 mg (500 mg of quinidine) in 10 ml of sterile water	

[a] Adapted from *CDC Guidelines for Treatment of Malaria in the United States* (23 March 2005), http://www.cdc.gov/malaria/diagnosis_treatment/treatment.html. Also see Table 26.17, chapter 36, and reference 49.

Primaquine can cause hemolytic anemia, especially in patients whose RBCs are deficient in glucose-6-phosphate dehydrogenase (G6PD). This deficiency is most common in African, Asian, and Mediterranean peoples. Patients should be screened for G6PD deficiency before treatment. Limited evidence suggests that G6PD-deficient patients may be safely treated with a single weekly dose of primaquine (0.75 mg/kg) for 8 weeks. Resistance is not known to occur at a dose of 0.5 mg/kg daily for 14 days: suspected resistant strains may be treated with this daily dose for 28 days. Relapse despite adherence to a full primaquine dose may be due to a poor metabolizer phenotype. Primaquine should not be used during pregnancy. It should be taken with food to minimize nausea and abdominal pain (49).

An increase in the incidence of malaria caused by *P. falciparum* is well recognized, and delays in diagnosis and subsequent therapy may result in complicated or fatal cases, particularly in nonimmune hosts. Some of the complications of *P. falciparum* malaria that have been discussed include cerebral malaria, renal failure, respiratory problems, disseminated intravascular coagulation, blackwater fever, and hepatic dysfunction. Death is directly related to the level of parasitemia and onset of complications, with significant mortality related to >5% parasitemia in spite of appropriate parenteral therapy and supportive care. In these situations, the role of exchange blood transfusion may be appropriate for the following reasons: (i) rapid reduction of the parasitemia, (ii) decreased risk of severe intravascular hemolysis, (iii) improved blood flow, and (iv) improved oxygen-carrying capacity. Powell and Grima have published an excellent review of the use of exchange transfusion for treatment of malaria (50).

Exposure to both placental malaria and maternal HIV infection increases postnatal mortality to a greater degree than does the independent risk associated with exposure to either maternal HIV or placental malaria infection alone. Therefore, malaria chemoprophylaxis during pregnancy could decrease the impact of HIV transmission from mother to infant (Table 26.17).

New Drug Development. Past approaches used to identify potential new antimalarials involved empirical screening of many natural plant extracts, as well as synthesis of new analogs of compounds known to have activity against microbes, tumors, and/or malarial parasites. Primary studies involve *in vitro* cultures and animal models; secondary and tertiary screening is then undertaken. Very few compounds are selected for clinical evaluation due to financial, compound formulation, stability, toxicity, or efficacy problems encountered in preliminary testing. New approaches involving computerized structure-based drug design and molecular modeling represent significant improvements. Molecular biology methods have become very important and widely used in this development process.

Drug candidates are currently being identified and developed for further testing. With the continued increase in the number of multiply drug-resistant strains of *P. falciparum*, these drug development efforts have resulted from renewed commitment and have been moved up the priority list. However, the process of antimalarial drug discovery, design, and testing is very long and complex, not to mention expensive. In the United States, a national average of 12 to 15 years and an investment of $200 million to $500 million per licensed drug are expended.

There seem to be inconsistencies between malaria control initiatives and actual policy development and implementation. Current consensus seems to be that a policy change is urgent when high-level resistance occurs in 40% or more of treated patients, when parasitological response is poor, and when the costs of treatment failures are higher

TABLE 26.16 Drugs used for the prevention of malaria[a]

Drug	Usage	Adult dosage	Pediatric dosage	Comments
Atovaquone/ proguanil[b] (Malarone)	Primary prophylaxis in areas with chloroquine-resistant or mefloquine-resistant *Plasmodium falciparum*	250 mg of atovaquone/ 100 mg of proguanil hydrochloride; 1 adult tablet orally daily	62.5 mg of atovaquone/25 mg of proguanil hydrochloride 5–8 kg: 1/2 peds tablet/day 9–10 kg: 3/4 peds tablet/day 11–20 kg: 1 tablet/day 21–30 kg: 2 tablets/day 31–40 kg: 3 tablets/day >40 kg: 1 adult tablet daily	Contraindicated in persons with severe renal impairment; should be taken with food or a milky drink; not recommended for children <11 kg, pregnant women, or women breast-feeding infants weighing <11 kg
Chloroquine phosphate (Aralen and generic)[c]	Primary prophylaxis in areas where chloroquine-sensitive *P. falciparum* is endemic	300 mg of base (500 mg of salt) orally once/ wk	5 mg/kg (base), 8.3 mg/kg (salt) orally once/wk, up to maximum adult dose of 300 mg of base	Safe to take during pregnancy, including first trimester; may exacerbate psoriasis; beginning 1–2 wks before travel, continuing weekly during stay and for 4 wks after leaving malarious zone
Doxycycline (many brand names and generic)[d]	Primary prophylaxis in areas with chloroquine-resistant or mefloquine-resistant *P. falciparum*	100 mg orally once/day	>8 years of age: 2 mg/kg orally/day up to maximum adult dose of 100 mg/day	Contraindicated in children >8 years of age; teeth may become permanently stained; may cause photosensitivity; contraindicated during pregnancy
Mefloquine (Lariam and generic)[a]	Primary prophylaxis in areas with chloroquine-resistant *P. falciparum*	228 mg of base (250 mg of salt) orally once/ wk (tablet 250 mg of salt)	5–10 kg: 1/8 tablet orally once/wk 10–20 kg: ¼ tablet once/wk 20–30 kg: ½ tablet once/wk 30–45 kg: ¾ tablet once/wk >45 kg: 1 tablet once/wk The recommended dose of mefloquine is 5 mg/kg of body weight once weekly. Approximate tablet fraction is based on this dosage. Exact doses for children weighing less than 10 kg should be prepared by a pharmacist.	Contraindicated in persons allergic to mefloquine and in those with active depression or a previous history of depression, generalized anxiety disorder, psychosis, schizophrenia, other major psychiatric disorders, or seizures. Not recommended for persons with cardiac conduction abnormalities. As of 2013, some authorities recommend its use for prophylaxis only as a drug of last resort (CNS toxicity).[e]
Primaquine (for primary prophylaxis)	An option for primary prophylaxis in special circumstances. Call Malaria Hotline (770-488-7788) for additional information.	30 mg base (52.6 mg salt) orally daily	0.5 mg/kg base (1.0 mg/kg salt) up to adult dose orally daily	Contraindicated in persons with G6PD[f] deficiency. Also contraindicated during pregnancy and lactation unless the infant being breast-fed has a documented normal G6PD level. Use in consultation with malaria experts.
Primaquine (for terminal prophylaxis)	Used for terminal prophylaxis to decrease the risk of relapses of *P. vivax* and *P. ovale* infection	30 mg of base (52.6 mg of salt) orally once/ day for 14 days after departure from the malarious area **Note:** The recommended dose of primaquine for terminal prophylaxis has been increased from 15 mg to 30 mg for adults.	0.6 mg/kg base (1.0 mg/kg salt) up to adult dose orally once/day for 14 days after departure from the malarious area **Note:** The recommended dose of primaquine for terminal prophylaxis has been increased from 0.3 mg/kg to 0.5 mg/kg for children.	Indicated for persons who have had prolonged exposure to *P. vivax* and *P. ovale* or both. Contraindicated in persons with G6PD deficiency. Also contraindicated during pregnancy and lactation unless the infant being breast-fed has a documented normal G6PD level.

(continued)

TABLE 26.16 *(continued)*

Drug	Usage	Adult dosage	Pediatric dosage	Comments
Atovaquone-proguanil (Malarone); self-treatment drug	Self-treatment drug to be used if professional medical care is not available within 24 h; medical care should be sought *immediately* after treatment	4 tablets (each dose contains 1,000 mg of atovaquone and 400 mg of proguanil) orally as a single daily dose for 3 consecutive days **Seek medical care immediately after treatment.**	<5 kg: not indicated 5–8 kg: 2 peds tablet/day × 3 days 9–10 kg: 3 peds tablet/day × 3 days 11–20 kg: 1 adult tablet/day × 3 days 21–30 kg: 2 adult tablets/day × 3 days 31–40 kg: 3 adult tablets/day × 3 days >40 kg: 4 adult tablets/day × 3 days **Seek medical care immediately after treatment.**	Contraindicated in persons with severe renal impairment (creatinine clearance, <30 ml/min). Not recommended for self-treatment in persons on atovaquone-proguanil prophylaxis. Not currently recommended for children weighing <11 kg, pregnant women, or women breast-feeding infants <11 kg.

[a] From Centers for Disease Control and Prevention, Travel Information (http://www.cdc.gov/malaria/travelers/) (49). peds, Pediatric.

[b] Take the first dose 1 to 2 days before travel to malarious areas; take doses daily, at the same time each day, while in the malarious area; take doses daily for 7 days after leaving such areas. Mefloquine should be taken on a full stomach, such as after dinner; most travelers have few if any side effects. However, side effects can include abdominal pain, nausea, vomiting, and headache.

[c] Take the first dose 1 to 2 weeks prior to arrival in the malarious area; continue once a week on the same day of the week during travel to malarious areas and for 4 weeks after leaving such areas. Side effects could include gastrointestinal disturbance, headache, dizziness, blurred vision, insomnia, and pruritus, but these symptoms usually do not require discontinuation of the drug.

[d] Doxycycline should be taken on a full stomach, such as after dinner; to minimize reflux, do not lie down for 1 h; can predispose women to vaginal yeast infections; should *not* be used in pregnancy, by children younger than 8 years, or by persons allergic to tetracyclines. Doxycycline can also cause sun sensitivity, usually manifested as an exaggerated sunburn reaction. Persons taking this drug should use sunscreen that absorbs long-wave UVA radiation.

[e] Nevin RL, *Int J Parasitol Drugs Drug Resist* **4**:118–125.

[f] G6PD, glucose-6-phosphate dehydrogenase.

than those of treatments with a newer drug. Improved cooperation is needed between the private and public sectors regarding overall delivery of health care relevant to malaria control issues (51).

Counterfeit Drugs for Malaria. Counterfeiting of drugs has now emerged as one of the most critical public health problems for malaria treatment in developing countries. During the past centuries, poor quality drugs surfaced in the 1600s with fake cinchona bark and in the 1800s with fake quinine. Although the problem of modern-day counterfeit drugs was first addressed in 1985 at a conference in Nairobi, the counterfeit market continues to grow and has become worldwide during the last decade. The World Health Organization (WHO) estimates that 50% of medicines available via the Internet are fake (52).

Counterfeiting of drugs is estimated by WHO at more than US$35 billion and represents more than 15% of the pharmaceutical market worldwide; unfortunately this percentage increases to more than 60% in developing countries (35 to 90% in Southeast Asia).

The situation is even worse in sub-Saharan Africa where the burden of malaria is the greatest. In 2001, WHO recommended that malaria-endemic African countries consider changing to artemisinin-derivatives as first-line treatment for malaria. However, adopting this new policy was difficult due to the high cost and relative shortage of the raw plant material source (*Artemisia annua*). Thus, the high cost and shortage of drugs led to the spread of fake artemisinin which places many thousands of African young children at risk. A large proportion of these fake antimalarials are imported from Asia. Also, locally produced antimalarials may be substandard or fake, with a name, label, and packaging that are different from the genuine drugs. Unfortunately, there does not seem to be an easy answer to this problem; however, recognizing the scope of the problem is certainly the first step.

Overall Patient Care. It is very important to remember that patients who present to a medical clinic, office, etc., that does not have expertise in tropical medicine tend to receive suboptimal treatment. Malaria may not be recognized, and the diagnosis may be delayed until it is no longer possible to save the patient. Improvements in obtaining the patient history, symptom recognition, presumptive diagnosis development, and treatment of malaria are mandatory to prevent severe illness and death among travelers. These patients tend to be immunologically naive, have never been exposed to malaria before, and have no residual antibody. Without the benefit of immunologic protection, they present with very low parasitemias but may be seriously ill. Their illness will mimic many other conditions, adding to the lack of recognition of a possible malaria infection. Unfortunately, this situation often occurs in

TABLE 26.17 Current issues related to maternal malaria, transmission, pathogenesis, and therapy options

Issue	Comments	Reference(s)
Naturally acquired *Plasmodium knowlesi* in human (Thailand)	The occurrence of simian malaria in humans emphasizes the possible importance of wild primate populations in malaria-endemic and disease transmission areas.	119
Perinatal mother-to-child transmission (MTCT) of HIV	Placental malaria controlled at low density may cause increase in immune responses that protect against MTCT. Uncontrolled, high-density malaria may disrupt placenta structure and generate antigen stimulus to HIV replication, thus increasing the risk for MTCT.	120
Chloroquine resistance in *P. vivax*	Chloroquine has been the drug of choice for *P. vivax* since 1946. No genetic mutations have been linked to chloroquine resistance in *P. vivax* infections. Some recommend mefloquine as alternative; daily doxycycline is also an option when combined with chloroquine. Malarone plus primaquine is the only available therapy with proven efficacy (>90%).	121
Chloroquine resistance in *P. falciparum*	By 1973 in Thailand, chloroquine replaced by a combination of sulfadoxine and pyrimethamine (SP); >10 African countries also switched their first-line drug to SP. In 1985 SP was replaced by mefloquine; resistance to mefloquine led to introduction of artemisinin as combination drug in the mid-1990s. New drugs cost more, have more side effects, take longer time for cure, and have more compliance problems than chloroquine. Another option would be to combine SP (better parasitologic response than chloroquine) with chloroquine (more rapid clinical response).	122, 123
Atovaquone-proguanil resistance in nonimmune North American traveler to West Africa (*P. falciparum*)	Atovaquone-proguanil is a useful agent for treating uncomplicated *P. falciparum* malaria (oral, short course, low side effects). PCR and sequence analysis confirmed the acquisition of a point mutation in the cytochrome *b* gene of the recrudescent isolate (known to confer high-level resistance to atovaquone by reducing the binding affinity of atovaquone). Also, in this case, suboptimal therapy may have played a role in the emergence of resistance.	124
Rapid elimination of asexual forms of *P. falciparum* (most effective way to prevent development of gametocytes)	Primaquine (mefloquine-primaquine) is not effective in the eradication of gametocytes in the primary infection and in the prevention of subsequent gametocytemia compared with artesunate therapy (mefloquine-artesunate). Recrudescent infections lead to subsequent gametocytemia, which drives drug resistance.	125
Distinguishing *P. falciparum* treatment failures from reinfections: PCR genotyping	Study in Tanzania: all samples contained multiple *P. falciparum* infections; primary and recrudescent parasites analyzed by PCR; some of the recrudescing parasites originated from new infections.	126
Fake antimalarials in Southeast Asia (major impediment to malaria control)	Myanmar, Lao People's Democratic Republic, Vietnam, Cambodia, Thailand: 53% of tablet packs labeled as "artesunate" contained no artesunate; 9% of mefloquine samples contained <10% of the expected amount of active ingredient.	127
Recrudescence in artesunate-treated *P. falciparum* cases (depends on parasite burden, not on parasite factors)	Artemisinin derivatives are first-line drugs in Thailand; no documented resistance to artemisinin. Patients with admission parasitemias of >10,000/μl had a 9-fold higher likelihood of recrudescence than did patients with lower parasitemias; recrudescence not due to resistance but to admission parasitemia.	128

areas where malaria is not endemic and thus may be the last causative agent considered.

Epidemiology and Prevention

Malaria is primarily a rural disease and is transmitted by the female anopheline mosquito. There are great variations in vector susceptibility to infection with the parasite, with many variations being related to differences in parasite strain. Even when the vector is present in an area, an average number of bites per person per day must be sustained or the infection gradually dies out. This critical level can be influenced by a number of factors, including the vector preference for human blood and habitation and the duration of infection in a specific area. Once an area is clear of the infection, there may also be a drop in population immunity, a situation that may lead to a severe epidemic if the infection is reintroduced into the population (53) (Table 26.18).

Immunity. There are some genetic alterations in the RBCs that confer natural immunity to malaria. Changes in the RBC surface interfere with attachment and invasion of merozoites. Changes in hemoglobin or intracellular enzymes interfere with parasite growth and multiplication (Table 26.19).

Duffy antigen-negative RBCs lack surface receptors for *P. vivax* invasion. Many West Africans and some American blacks are Duffy antigen negative, which may explain the low incidence of *P. vivax* in West Africa. In other areas of Africa, *P. vivax* is much more prevalent.

Partial resistance is seen in individuals with the sickle cell trait and in those with sickle cell anemia. Resistance is related to the sickling of hemoglobin S (HbS)-containing RBCs. Other factors include the formation of deoxyhemoglobin aggregates within the cells, the loss of potassium from sickled cells, and the fact that the parasites actually cause HbS cells to sickle.

Resistance to *P. falciparum* is also seen in G6PD-deficient cells. Partial immunity to malarial infection is seen in areas where malaria is endemic when HbC, HbE, β-thalassemia, and pyruvate kinase deficiencies exist.

Infants are also relatively immune to malarial infections during the first year of life as a result of the presence of a large percentage of HbF, passive immunity from maternal antibodies, and diets deficient in *p*-aminobenzoic acid.

Both cellular and humoral immunities play a part in protection. However, immunity is species specific and in some cases even strain specific. It has also been noted that acute malarial infections can cause immunosuppression. Actual impairment of immune responses to vaccination after acute malaria has been documented.

Insecticide-Impregnated Bed Nets and Clothing. Insecticide-impregnated bed nets have been widely used with various degrees of success. Important factors for the success of any bed net program include insect susceptibility to the particular insecticide used (often pyrethroids), high coverage with impregnated bed nets, high malaria incidence, good community participation at an acceptable sustained level, high mosquito densities when people go to bed, and a high proportion of *P. falciparum.* Bed net impregnation appeared to be somewhat more effective than DDT spraying (Fig. 26.21) (54–56).

The use of permethrin-impregnated clothing has also been evaluated and has been found to reduce the incidence of both malaria and leishmaniasis by a large percentage in the absence of any other interventions. In addition to use by the military (57), permethrin-impregnated clothing for travelers has a number of advantages. First, this insecticide is not a repellent but actually kills mosquitoes, flies, and ticks on contact. Also, impregnated clothing fibers remain effective even after 3 months of repeated machine washing. Apparently, this approach, in which the clothing is soaked with the appropriate solutions, works better than use of an aerosol spray. It is recommended that travelers treat two sets of clothing to wear in high-risk situations. Permethrin liquid is available in the United States as Permanone.

Vaccines. The development of techniques for *in vitro* culture of *P. falciparum* has enhanced the ability to study parasite metabolism and mechanisms of attachment and invasion. Other studies involving genetic engineering techniques and serologic immunofluorescence tests have led to the development of genetic manipulation of *Escherichia coli* to produce proteins that are identical to the receptor protein on the parasite necessary for RBC attachment. With the use of these and other, newer techniques, progress toward vaccine production may lead to effective protection against malarial infections. Increased funding through private and public partnerships has led the industrial sector to become more involved in vaccine research.

The Perfect Vaccine. A perfect malaria vaccine will induce immune responses to every stage of the complicated life cycle of *Plasmodium*. Different and distinct immune mechanisms operate against these different stages in the life cycle. Therefore, a multistage vaccine must be a multi-immune response vaccine. Development of these types of vaccines is required to reduce significantly the increasing numbers of malaria infections and deaths seen each year (58).

Pre-erythrocytic stage as vaccine target. A pre-erythrocytic stage vaccine would prevent sporozoites from invading the liver or prevent liver-stage parasites from developing to maturity and releasing infective merozoites, or both. This type of vaccine would prevent disease (absence of RBC cycle and clinical symptoms) and the transmission of malaria (no gametocyte formation).

Erythrocytic stage as vaccine target. The asexual RBC stage vaccine would prevent or reduce morbidity or mortality by parasite load reduction. Neutralizing antibodies to parasite products (released during schizont rupture) or inhibiting cytoadherence of infected RBCs in severe forms of malaria would thus reduce morbidity and mortality.

Sexual stage as vaccine target. A vaccine designed to induce immune responses to the sexual-stage antigens would provide no protection to the infected patient but would dramatically limit or eliminate transmission of the parasite within the community (it would destroy gametocytes, prevent fertilization, and prevent development of the parasite within the mosquito).

Multistage, multivalent, multi-immune response vaccine. One of the main advantages of the multistage vaccine is its ability to combat parasite variation. Organism variants that evade the immune mechanisms operating at one of the early stages in the life cycle would most probably be susceptible to one of the later-stage immune mechanisms. Antigenic characteristics of the parasite vary with the life cycle stages, and most antigens are not expressed during all stages but may be present only during a single stage. This mandates either total removal of the parasite in one form within the

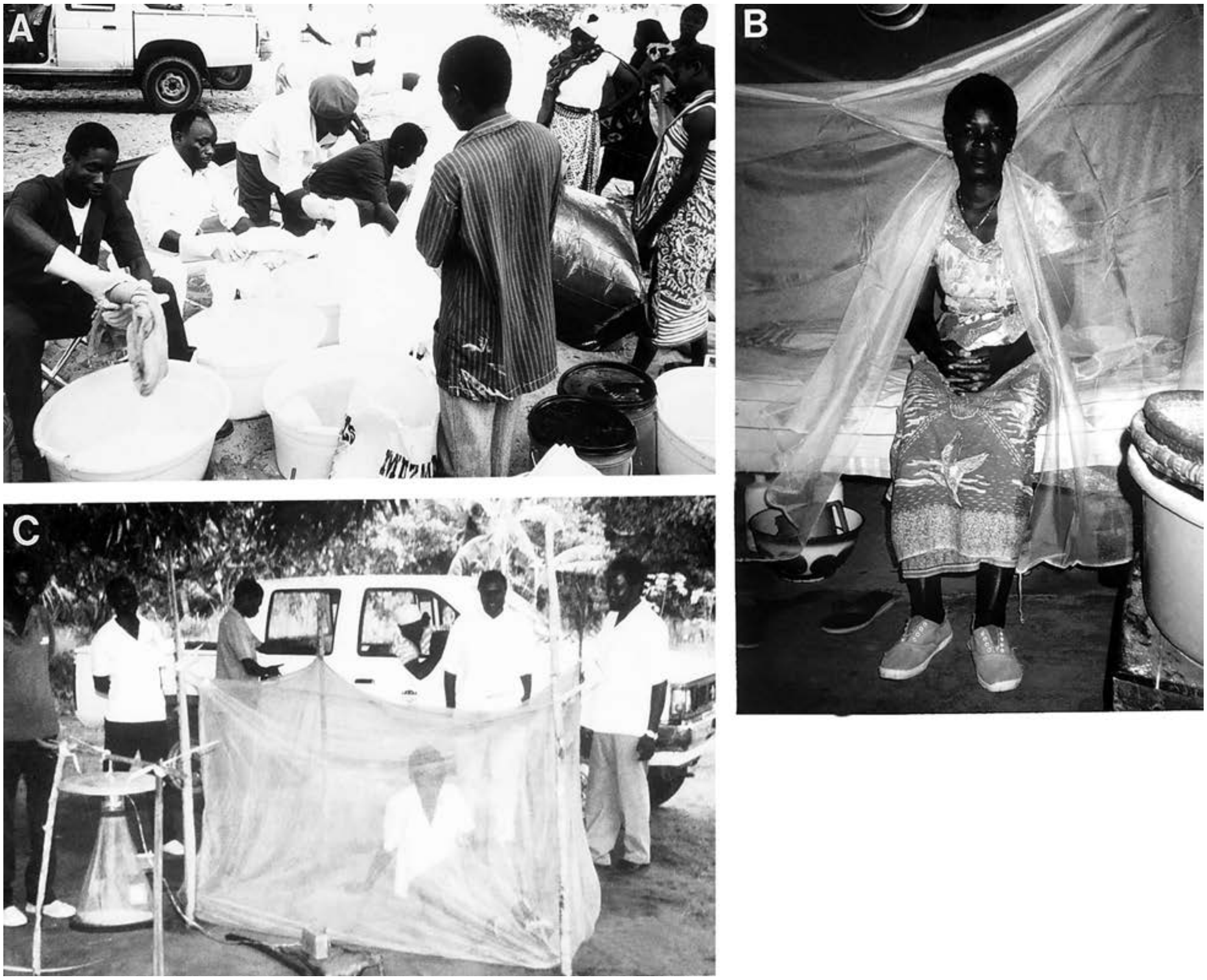


Figure 26.21 Bed nets used in malaria prevention. (A) Nets being dipped in a suspension of insecticide following purchase. For people to appreciate the value of the insecticide treatment, it is important for them to see the process. The nets are wrung out, placed in a plastic bag, and returned to the owner. Later, in the house, the net is laid directly on the bed and allowed to dry out. This must be done indoors to avoid direct exposure to the sun, which degrades the insecticide. (B) Net in place in the home. The occupant of the house in the Bagamoyo BedNet Project, Tanzania, is preparing for rest. The net is a rectangular design, which provides a better ventilating effect than the conical variety. (C) Rectangular bed net being set up outdoors to enable the collection of mosquitoes by the Centers for Disease Control and Prevention Light Trap suspended on the left. Nets for this use are not treated with insecticide. Mosquitoes attracted to the person sleeping under the net are attracted to the light and enter the trap. In the morning, the trap is removed and the collection will indicate which mosquito species are hunting outdoors for blood meals. Similar collections can be made indoors. (Courtesy of Clive Shiff, Johns Hopkins University, with permission.) doi:10.1128/9781555819002.ch26.f21

life cycle or partial removal of various stages, with the overall result being parasite elimination (59).

For a malaria vaccine to induce optimal and sustainable protection, both cellular and humoral responses will have to be elicited against multiple targets from the different life cycle stages. It appears that global malaria vaccine development will move forward on several fronts, hopefully leading to effective products available for clinical use (58, 59).

Overall Control of Malaria. The major determinants related to malaria transmission include (i) the parasite, with its many biological options and genetic diversity; (ii) the vector, with tremendous differences in behavior and transmission capability; (iii) the human host, with biological, behavioral, political, and social ramifications; and (iv) the environment, with tremendous changes such as altitude, temperature, rainfall, and global climate change (Table 26.20). Relevant concepts for the control of mosquito transmission of human

TABLE 26.18 Malaria epidemics in recent years: climatic change, population displacement, control policy failures, drug resistance[a]

Geographic area	Comments
Central America	Agricultural development, irrigation changes, colonization, insecticide resistance have led to overall increase in malaria
South America	
Amazon rain forest	New settlement and mining have led to >500,000 cases/year in Brazil (>50% of malaria cases in the Americas) and 6,000–10,000 deaths
Peru	Prolonged rains, abnormally high temperatures, and long, humid summers have led to an increase in malaria
Africa	
Savanna and forest	Increasing chloroquine resistance; >50% of population is infected; malaria is main cause of death in children, killing 1 in 20 younger than 5 yr
Dry savanna and desert fringe	Increased rains and population movements have led to epidemics
Cities	Severe increased drug resistance, inadequate sanitation, overextended services, and increasing deaths in young adults are all factors
Burundi, Rwanda, neighboring countries	Mass refugee problem has led to worsening overall malaria situation
Botswana, Namibia, Swaziland, Zimbabwe	Exceptional rains and population movements have led to an overall increase in malaria
East African highlands and Madagascar	Cyclical epidemics related to meteorological changes, complicated by agriculture changes, deforestation, and population movements
Ethiopia	Repeated epidemics in highlands due to degradation of environment, drought and famine, and large-scale resettlement approaches
Azerbaijan, Iraq, Tajikistan, Turkey	Civil disturbances, population displacement, and intensified agricultural activities have led to an epidemic resurgence of malaria
Afghanistan	Interruption of control and displacement of populations due to war are responsible for 2 million to 3 million cases annually
South Asia: middle	Increased epidemics in tribal, forest, and hill areas; periodic epidemics in dry lands of northwestern India and Sri Lanka (pooling of river sources, environment conducive to vector breeding); over 2.5 million cases/yr
Cambodia, Lao People's Democratic Republic, Myanmar, Thailand, Vietnam	500,000 cases/yr; rapidly increasing risk in frontier areas of economic activity, often including illicit mining and civil unrest; most severe drug resistance in the world
Papua New Guinea, Philippines, Solomon Islands, Vanuatu	300,000 cases/yr, released to colonization of new areas

[a] Adapted from the WHO/CTD Health Map, 1997, and reference 53.

malaria parasites are listed in Table 26.21; examination of this aspect of malarial transmission control provides an excellent example of the complexities that exist.

Another critical aspect of control involves the safety of the blood supply. A number of factors impact the blood supply worldwide, including government policies and regulations, professional society standards, the donor pool (voluntary and/or paid), the ability of health care personnel to order blood when necessary, infectious-disease screening, posttransfusion adverse effects monitoring, and product-related side effects (60). A number of constraints will continue to affect the development of a global strategy for the control of malaria (Table 26.22).

Babesiosis

When blood parasites are discussed, malaria is usually considered the most important and well-known infection. However, there is another organism that also causes human infection. Recognition of the spectrum of disease caused by organisms of the genus *Babesia* has recently resulted in an increase in the number of cases reported. Increased interest has also been generated because the infection can be transmitted via transfusion, can cause serious illness, particularly in compromised patients, and is difficult to treat (61).

The *Babesia* blood parasite was first discovered in cattle in 1888, and in 1893 it was recognized as the cause of Texas cattle fever transmitted by ticks (48). Cases of babesiosis have been documented worldwide, and several outbreaks in humans have occurred in the northeastern United States, particularly in Long Island, Cape Cod, and the islands off the East Coast (61). Although there are many species of *Babesia*, *Babesia microti* is the cause of most human infections in the United States.

A case reported from California occurred in a 24-year-old man who had been splenectomized in 1985 for an immune system-mediated thrombocytopenia and who was a U.S. Army officer stationed at Fort Ord in Monterey County. This is the third reported case of presumed indige-

TABLE 26.19 Host genetic factors in resistance to malaria[a]

Factor[b]	Comments
Hemoglobin disorders	
Sickling disorders	Includes sickle cell disease (SS), heterozygous states for sickle cell gene and those for hemoglobin C (SC) or β-thalassemia (S-thal); carrier state for sickle cell gene is AS; widely distributed throughout tropical Africa, parts of the Mediterranean, the Middle East, and central India; hemoglobin S confers protection against *P. falciparum* malaria (the parasite cannot complete life cycle due to cell sickling and destruction; reduced oxygen levels result in diminished parasite growth; reduced rosetting of RBCs in sickle carriers; protection also related to hemoglobin structure and parasite inability to metabolize)
Hemoglobins C and E	Data relating to selective advantage against malaria less convincing than for hemoglobin S; reduced parasite invasion and impaired growth have been documented; data for hemoglobin E have shown inconsistent results
Hemoglobin F	Growth of *P. falciparum* reduced in presence of hemoglobin F, thought to be due to hemoglobin itself rather than RBC properties; higher levels of hemoglobin F during first year of life might offer protection (newborn infants and adults with persistent fetal hemoglobin production)
Thalassemia (α and β)	Distribution of β-thalassemia coincides with that of malaria; 70% reduction in clinical malaria and 50% reduction in risk; although not seen in all geographic areas, in Papua New Guinea high protective effect of homozygous state for β-thalassemia against complications of *P. falciparum* malaria; babies under 2 yr (homozygous for α^+-thalassemia) had higher frequency of both *P. vivax* and *P. falciparum* but were resistant from 2 yr of age
Erythrocyte polymorphisms	
Glucose-6-phosphate dehydrogenase deficiency	Both female heterozygotes and male hemizygotes have reduced risk (around 50%) of developing severe malaria
Duffy-negative RBCs	Resistant to invasion by *P. vivax*
Ovalocytosis	Patients subject to severe malarial infection with high parasitemias; however, there is strong protection against cerebral malaria; structural change in RBC membrane interferes with binding of infected RBCs to vascular endothelium
Immunogenetic variants	
HLA genes	Each protective HLA type associated with 40–50% decrease in risk; these HLA types are common
HLA class I	HLA-A, -B, -C determine specificities of $CD8^+$ T cells (cytotoxic, major role in defense against intracellular pathogens); HLA-B35 frequency reduced in children with cerebral malaria and those with severe malarial anemia
HLA class II	HLA-DR, -DQ, -DP determine specificities of $CD4^+$ T cells that secrete cytokines and provide T-cell help for antibody production and action of other T cells; HLA-DRB1*1302–HLA-DQB1*1501 frequently reduced in children with severe malarial anemia
Cytokine, other immune response genes	
TNF	Major mediator of malaria fever; TNF is increased in children with cerebral malaria and markedly so in children with fatal cerebral malaria
MBL	Deficiency may be associated with increased susceptibility to infectious diseases; effect on malaria may be small to none
CD35	Also called complement receptor 1 (CR1); plays a role in rosetting; African variant of CR1 may protect against severe malaria

[a]Adapted from reference 129.

[b]HLA, human leukocyte antigen; TNF, tumor necrosis factor; MBL, mannose-binding lectin.

nous transmission of babesiosis in patients in California, all of whom had been previously splenectomized.

Over the past few years, one patient from Washington and four from California were identified as being infected with a similar protozoal parasite, very similar in morphology to *Babesia* species; the Washington isolate was designated WA1. All four patients had undergone splenectomy, two as a result of trauma and two for medical reasons. Two of the four patients had complications, and one died. Piroplasm-specific nuclear small-subunit ribosomal DNA was recovered from the blood of the four patients by PCR amplification. Genetic sequence analysis showed that the organisms were almost identical. Phylogenic analysis showed that this strain is more closely related to a known canine pathogen (*B. gibsoni*) and to *Theileria* species than to most members of the genus *Babesia*. Serum from three of the patients was reactive to WA1 but not to *B. microti* antigen. It now appears that there is a newly identified *Babesia*-like organ-

TABLE 26.20 Epidemiology of malaria[a]

Characteristic	Stable malaria	Unstable malaria
Transmission pattern	Occurs throughout the year; uniform intensity; pattern repeated yearly	Seasonal transmission intensity variable; dramatic epidemics likely
Community immunity	Strong resistance due to intense transmission	Reduced resistance due to low-level and variable transmission intensity
Age group affected	Mainly young children	All age groups
Control	Difficult	Easier
Major determinants relevant to malarial transmission	Parasite—different biology, genetic diversity Vector—air temperature, relative humidity, breeding sites, density, feeding times, resting places, life cycle Human host—genetic factors, acquired immunity, general health status, behavior patterns, political/social factors Environment—altitude, temperature, rainfall, flow rate of rivers and streams, global climate change, colonization, group migrations, agricultural and building projects, deforestation	

[a] Adapted from reference 107.

ism which causes clinical infections in the western United States. The spectrum of illness ranges from asymptomatic infection to fulminant, fatal disease (61). The first case of transfusion-transmitted babesiosis with the WA1 type has been reported from Washington State.

Studies in New York State indicate evidence of exposure to five tick-borne pathogens, including *B. microti*, in a high-risk population in the northeastern United States. There was also evidence of possible dual infection in some patients (62). In the same area, a cluster of transfusion-associated babesiosis cases was traced to a single asymptomatic donor; six patients were exposed, and three of these developed parasitemia (63). Environmental conditions necessary for the transmission of *B. microti* now appear to be present in areas of New Jersey, and physicians in central New Jersey should be aware of this potential threat.

A total of 10 cases of babesiosis have been reviewed, most of which were probably acquired in northwestern Wisconsin. Of 10 cases, 3 were fatal and occurred in elderly patients, who died after experiencing complications (64). These cases also serve to emphasize the importance of this potentially fatal zoonosis, which is not limited to the northeastern United States. In another case from western Wisconsin, a woman who had not traveled outside of Wisconsin was hospitalized and found to be infected with both *B. microti* and *Borrelia burgdorferi* (65).

Babesia spp. are grouped into the small *Babesia* spp. (1.0 to 2.5 µm), which include *B. gibsoni*, *B. microti*, and *B. rodhaini*, and the large *Babesia* spp. (2.5 to 5.0 µm), which include *B. bovis*, *B. caballi*, and *B. canis*. These phenotypic classifications are, for the most part, consistent with genetic characterization based on nuclear small-subunit ribosomal DNA sequences. The small *Babesia* spp. are more closely related to *Theileria* spp. than are the larger organisms. The one exception is *B. divergens*, which appears small on blood smears but is genetically related to the large *Babesia* spp. The two primary pathogens of humans are *B. microti* and *B. divergens*, along with the unnamed species WA1 (Washington), CA1 (California), and MO1 (Missouri) (66).

Life Cycle and Morphology

These organisms have at least three stages of reproduction within the life cycle: (i) gamogony (formation and fusion of gametes inside the tick gut), (ii) sporogony (asexual reproduction in salivary glands), and (iii) merogony (asexual reproduction in the vertebrate host). Infection is transmitted by several species of ticks in which the sexual multiplication cycle occurs. Several thousand sporozoites can be found in the dermis surrounding the tick's mouth during the final hours of attachment and subsequent feeding. In addition to tick bites, transmission can also occur via blood transfusion or vertical transmission from mother to child, confirmed by detection of *Babesia* DNA in placenta tissue (67).

When a tick takes a blood meal, the infective forms are introduced into the human host (Fig. 26.22). The organisms infect the RBCs, in which they appear as pleomorphic, ring-like structures (Fig. 26.23). They tend to be somewhat pear-shaped within the RBCs (piroplasms). They resemble the early trophozoite (ring) forms of malarial parasites, particularly *P. falciparum*. The organisms measure 1.0 to 5.0 µm, the RBCs are not enlarged or pale, and the cells do not contain stippling. Malarial pigment is never seen. The early form contains very little cytoplasm and has a very small nucleus. In mature forms, two or more chromatin dots may be seen. Occasionally, a tetrad formation, referred to as a Maltese cross, is seen (Fig. 26.23). While ring forms may be seen outside the RBCs in *Babesia* infections, ring forms outside of the RBCs are rare in *Plasmodium* infections.

TABLE 26.21 Relevant concepts for the control of mosquito transmission of human malaria parasites[a]

Concept	Discussion	Control relevance
Only mosquitoes in the genus *Anopheles* support the sporogonic development of human malaria parasites.	A high degree of specificity exists between mosquito species and malaria parasites. Approximately 50 of the 500 *Anopheles* species mosquitoes are competent vectors of human malaria.	It is important to correctly identify vector species in nature and to target vector control to coincide with blood-feeding and resting behaviors of the vector species.
Infected humans carrying gametocyte-stage parasites are the "reservoir of infection."	The probability that mosquitoes will become infected depends on the number and quality of gametocytes present and ingested during blood-feeding.	Most antimalarials have no direct effects on gametocytes; thus, treated individuals may remain infectious for the mosquito vectors.
The rate of *Plasmodium* development in the mosquito depends primarily on temperature.	The rate of development from ingested gametocytes to sporozoites in the salivary glands depends on temperature. Temperature also determines mosquito survival and the probability of mosquito infection.	These relationships play a large role in parasite survival and parasite growth, affecting both the probability and intensity of transmission.
A female mosquito can remain infective for life.	The female anopheline mosquito is capable of transmitting sporozoites during each consecutive blood-feeding instance, occasionally to multiple individuals during each feeding cycle.	Mosquito control has multiple parameters for consideration.
Epidemiology of human infection associated with vector populations	Worldwide patterns of epidemics are associated with environmental and climatic factors that influence vector distribution, abundance, and blood-feeding behavior.	Personnel knowledgeable about environmental issues and the relationship between the environment and vector control are vital.
Parasite transmission intensity depends on both the numbers of biting vectors and the proportion of vectors carrying sporozoites in the salivary glands.	Intensity of transmission depends on the entomological inoculation rate (EIR), which is the product of the biting rate and the proportion of mosquitoes with sporozoites. EIRs are expressed in terms of average numbers of infective bites per person per unit of time.	EIRs in Africa range from 1 to >1,000 infective bites/yr. EIRs of >5 bites/yr are associated with malaria prevalence rates of >70% in human populations.
Both the frequency of sporozoite inoculation and dose per infectious bite are important determinants of human infection.	Sporozoites inoculated by only a single mosquito can cause human malaria and serious illness, even death.	In areas of Africa where residents are exposed to <5 bites/yr, some of the highest incidences of severe disease can be seen.
Marked reduction in transmission intensity is required to lower the prevalence of human malarial infection.	An infection acquired by a single individual can be transmitted via mosquitoes to many uninfected individuals in a short time.	In some areas, EIR reductions of >95% are required before the malaria prevalence drops.
From a public health point of view, there are no acceptable levels of malaria transmission.	Multiple factors are required for control efforts to be successful, including an integrated approach: drug therapy, vector control, and public health education at the community level.	Future efforts will include vaccines, new antimalarial drugs, and better vector control means. Public health will emphasize "control" rather than "eradication."

[a] Adapted from reference 130.

Clinical Disease

The majority of babesiosis cases in the United States are caused by *B. microti* and occur in the Northeast and upper Midwest. A small number of infections caused by *B. duncani* and *B. duncani*-like organisms have been reported on the West Coast from California to Washington State. Also, *B. divergens*-like organisms have been reported in Kentucky, Missouri, and Washington State. In Europe, almost all reported cases have been due to *B. divergens*, and a few have been caused by the EU1 species, now called *B. venatorum* (68). Sporadic cases of babesiosis have also been reported in Asia, Africa, Australia, and South America. The symptoms in human babesiosis are quite variable, ranging from asymptomatic to intense malaria-like episodes resulting occasionally in death. Most often, symptoms are nonspecific (fever, headache, and myalgia).

It is suspected that the frequency of babesiosis in the United States is greater than the number of cases reported. Between 1970 and 1991, 136 cases were reported in New York State, while between 1969 and 1998, 160 cases were reported in Nantucket. Babesiosis is usually a self-limiting disease; there are probably also asymptomatic and undiag-

TABLE 26.22 Global strategy for malaria control: constraints[a]

Problem	Comments
Misleading assumptions: Malaria control is a vertical program Existing tools will not allow impact on morbidity and mortality Malarial control is sole responsibility of health sector Malaria in Africa is uniform and can be controlled by "magic bullet"	Misconceptions have led to reduced political and financial commitment at local, national, and international levels. Accelerated control programs have not been achieved due to lack of funding.
Shortage of people with appropriate knowledge	Little knowledge and understanding of malaria epidemiology and planning/management of control efforts. Peripheral health services are not equipped for correct and adequate diagnosis and treatment. Very few countries have operational training programs in place.
Drug resistance; shortage of antimalarial drugs	Complicates the development of national antimalarial drug policies and provision of adequate disease management. Poor-quality antimalarial drugs; no mechanisms to ensure quality, availability, and rational use of affordable and effective drugs.
Poor collaboration	Inter- and intracollaborative efforts within and among countries are poor. Surveillance systems and information exchange mechanisms are limited; improvement is required for successful implementation.
Limited commitment from private sector	New links between research and development and malaria control needs are minimal; limited commitment to development of new tools for control. However, various initiatives aiming to develop malaria clinical research have recently been launched. Donors are public or international (Global Fund, Roll Back Malaria Initiative, National Institutes of Health [NIH], European Developing Countries Clinical Trial Partnership [EDCTP] program), as well as private (Bill & Melinda Gates Foundation). These substantial funds should enhance the research of new antimalarial drugs and large-scale, adequately designed trials.

[a] Adapted from reference 53.

nosed carriers. The mortality rate has been reported at about 5% (61).

However, infections caused by *B. divergens* in Europe are far more serious, with a mortality rate of 42%. Fatal cases of *B. divergens* have been reported in France, Britain, Ireland, Spain, Sweden, Switzerland, the former Yugoslavia, and the former USSR (Table 26.23). In two patients from Austria and Italy, the DNA sequences were identical; however, in phylogenetic analysis, the organism clusters with *B. odocoilei*, a parasite of white-tailed deer that is related to *B. divergens* (69). Although human babesiosis is relatively new to Japan, it appears that a new type of *B. microti*-like parasite, the Hobetsu type, is the major type among Japanese wild rodents.

***Babesia microti*.** Most cases have been reported from seven states (Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, and Wisconsin). The incubation period may last from 1 to 9 weeks and clinical features are similar to those of malaria. The severity is variable depending on the immune status of the host, ranging from an asymptomatic infection to a severe life-threatening disease. Severe disease generally occurs in patients over the age of 50 years or with splenectomy, malignancy, HIV, or immunosuppressive medication. Although headache and lethargy are common, babesiosis is rarely associated with specific neurological problems in humans. Diminished consciousness and/or coma are rare complications that are associated with severe and often fatal disease. Most patients with *B. microti* infections develop mild to moderate flu-like illnesses characterized by malaise and fatigue that include the following symptoms: rash, fever, chills, sweats, headache, arthralgia, myalgia, anorexia, cough, or nausea. Rarely, mild splenomegaly or hepatomegaly may be seen. Clinical symptoms may last for weeks to months, but rarely for more than a year.

In the first four human cases (before 1969) within the United States, the patients had been splenectomized and three died as a result of the infection. Since then, other cases have been found in patients with intact spleens. In the patients from Nantucket Island, Massachusetts, who were not splenectomized, symptoms began 10 to 20 days after a tick bite and continued for several weeks. The symptoms started with general malaise, which was followed by fever, shaking chills, profuse sweating, arthralgias, myalgias, fatigue, and weakness. Hepatosplenomegaly was present, and five patients had slightly elevated serum bilirubin and transaminase levels as a result of hemolytic anemia.

During the summer of 1980, six patients from Shelter Island and eastern Long Island were diagnosed as having babesiosis caused by *B. microti*. Symptoms lasted for 19 to 24 days in five patients and included fever, shaking chills,

TABLE 26.23 Similarities and differences in *Babesia* spp.[a]

Species[b]	Geographic location	Vector	Incubation period	Disease	Comments
Babesia divergens	Europe; relatively rare, but 42% mortality	*Ixodes ricinus*	1–3 wk	Sudden appearance, hemoglobinuria followed by jaundice; shock-like picture	Most patients splenectomized (frequent contact with cattle); bovine pathogen; same vector for Lyme disease
EU1 (*B. divergens*-like) *B. venatorum*	Italy, Austria	Not known, but probably *I. ricinus*	One patient had possible tick exposure 2 wks prior to symptoms	Fever, chills, confusion, jaundice	Both patients splenectomized; organism most closely related to *B. odocoilei* (infects white-tailed deer)
Babesia divergens-like	Washington	Not known	Not known	Asplenic 82-year-old male with high parasitemia (41.4%) and multiorgan dysfunction including renal failure; survived	Patient's serum reacted to *B. divergens* but not to *B. microti* or WA1
Babesia microti	United States: northeastern and Great Lakes regions; range probably expanding to include as far south as New Jersey, parts of Europe, and Japan; mortality rate for clinically apparent infections, 5%	*Ixodes scapularis* (*I. dammini*)	1–6 wk; up to 3 mo	Most cases are mild and resolve on their own; most severe cases have fulminating malaria-like infection; malaise, chills, myalgia, anemia, fatigue, fever; hepatomegaly and splenomegaly may be present; hemolytic anemia several days to few months (more common in elderly or asplenic individuals)	Age range 3 wk to 86 yr; most within 50–60 yr; most are normosplenic; Japanese strains appear to be *B. microti*-like
Babesia duncani WA1	California, Washington	Not known, but *Dermacentor variabilis*, *Ornithodoros coriaceus*, and *Ixodes pacificus* found in areas where WA1 found	Not known	Splenectomized patients	Related to canine pathogen, *B. gibsoni*, but WA1 does not seem to infect dogs; highly virulent in hamsters
Babesia sp. CA1	California	Not known	Not known	Splenectomized patient; 2 patients had complicated courses, and 1 died	This strain is more closely related to *B. gibsoni* and to *Theileria* species
Babesia sp. MOI	Missouri	*Ixodes dentatus*	Not known	Parasitemia in 3–4%; systemic lupus erythematosus, diagnosed in 1979, taking prednisone; splenectomy in 1979	*B. divergens*-like; 72-year-old asplenic male
PB-1	Baboon colonies	Not known	Not known	Potential problems with organ xenotransplantation to human recipients	Most closely related to *B. microti*; may be organism previously described as *Entopolypoides*; issues include prevention of potential zoonotic transmission

[a] Adopted from references 61, 64–68, 70–72, and 131.

[b] CA, California; EU, European Union; MO, Missouri; WA, Washington.

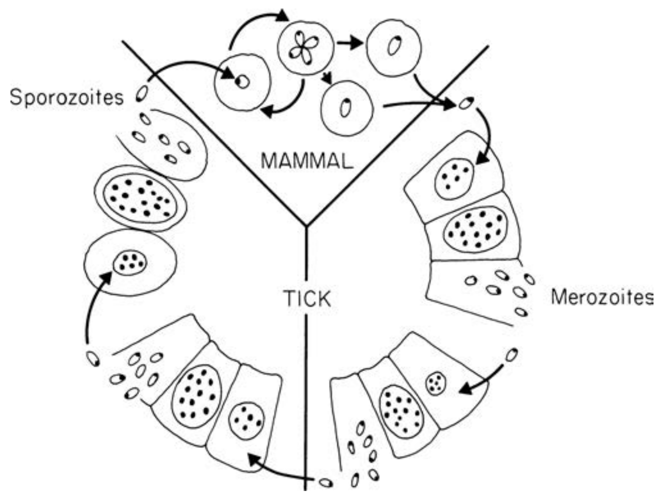


Figure 26.22 Life cycle of *Babesia* spp. (Illustration by Gwen Gloege.) doi:10.1128/9781555819002.ch26.f22

dark urine, and headache, as well as anorexia, malaise, and lethargy. The one patient in the group who had been splenectomized also had the most severe illness. The parasitemia could not be detected in one patient, was 5% in four of the six, and was 80 to 90% in the splenectomized patient. Hamster inoculation produced a patent parasitemia in all cases.

By the end of 1991, 13 cases of babesiosis had been reported in Connecticut, this being the largest number of human cases reported on the mainland United States. Information from the patients indicated that 12 infections were probably acquired via tick bites and 1 was acquired from a blood transfusion. The ages of the patients ranged from 61 to 95 years for those with tick-acquired infections. Two patients died of active infections, and one died of chronic obstructive pulmonary disease. Indirect fluorescent-antibody titers ranged from 11,024 to 14,096, and five of eight patients had significant IgG or IgM titers (1,640 to 15,120) to *B. burgdorferi*. *B. microti* from the blood of 7 of 12 patients tested was isolated in Syrian hamsters. It is now clear that babesiosis is endemic in New Jersey, as well as other areas of the northeast (70).

Cases of congenital babesiosis share similarities, including asymptomatic maternal infection and development of fever, hemolytic anemia, and thrombocytopenia in the infant detected between 19 and 41 days after birth. All of the infants responded to antimicrobial drug therapy; three were treated with azithromycin plus atovaquone, the preferred treatment regimen for mild babesiosis. All infants required a blood transfusion because of severe anemia. The clinical signs and symptoms for these cases of congenital babesiosis are similar to those of congenital malaria in nondisease endemic areas (67).

***Babesia duncani* (WA1).** *Babesia* infections described in California are quite different from those seen in the northeastern United States with *B. microti*, where the infection is most often subclinical. Infections in California and Washington tend to present as a fulminant, febrile, hemolytic disease affecting splenectomized or immunosuppressed individuals. *B. duncani* n.sp. is a distinct species from other known *Babesia* spp. for which morphologic and sequence information is available (71). All reported cases of WA1 babesiosis have occurred in the Pacific coast region of the United States, suggesting that WA1 is limited to this geographic area. However, WA1 IgG was found in 27% of clinical sera sent to a large reference laboratory for WA1 IgG testing from across the United States over a 2-year period, implying that exposure to WA1 or a closely related organism occurs outside Pacific coast states (72).

The serum of an 82-year-old splenectomized man from Washington reacted to *B. divergens* but not to *B. microti* or WA1 antigens. This case emphasizes the point that unusual parasites may be detectable by blood film examination but not by serologic or molecular testing for *B. microti* or WA1-type parasites (66). This is important to remember, since new organisms similar but not identical to a number of the well-known *Babesia* spp. may be identified in patients in different parts of the world. Without the manual examination of blood films, these organisms may be missed.

***Babesia* sp. CA-type.** Four severe to fatal human cases with *Babesia* sp. CA-type have been reported in splenectomized patients from California. Although this organism is morphologically indistinguishable from *B. duncani*, it can be distinguished by genetic sequencing.

***Babesia* sp. MO1.** *Babesia* sp. MO1 is a rare cause of babesiosis in the United States. In 1992, this novel *Babesia* species was detected in a splenectomized patient in Missouri. Despite treatment, the patient died 13 days after diagnosis. Two additional cases of *Babesia* sp. MO1 in splenectomized patients, both nonfatal, have been reported in Kentucky and Washington. Initial phylogenetic analyses indicated that the *Babesia* sp. (MO1) was closely related to *B. divergens*, suggesting that this European parasite had been introduced into the United States; however, additional research proved that *Babesia* sp. MO1 is distinct from *B. divergens*.

***Babesia divergens*.** The most common cause of human babesiosis in Europe is *B. divergens*, but other zoonotic species include a *B. divergens*-like species from the Canary Islands, *Babesia* sp. EU1 (also called *B. venatorum*), and a *B. microti*-like species. Infection with *B. divergens* is associated with a high death rate in splenectomized or immunocompromised patients. In Europe, sporadic cases of babesiosis have also been reported in immunocompetent persons.

The incubation period is usually 1 to 3 weeks during which the patients complain of malaise and discomfort.

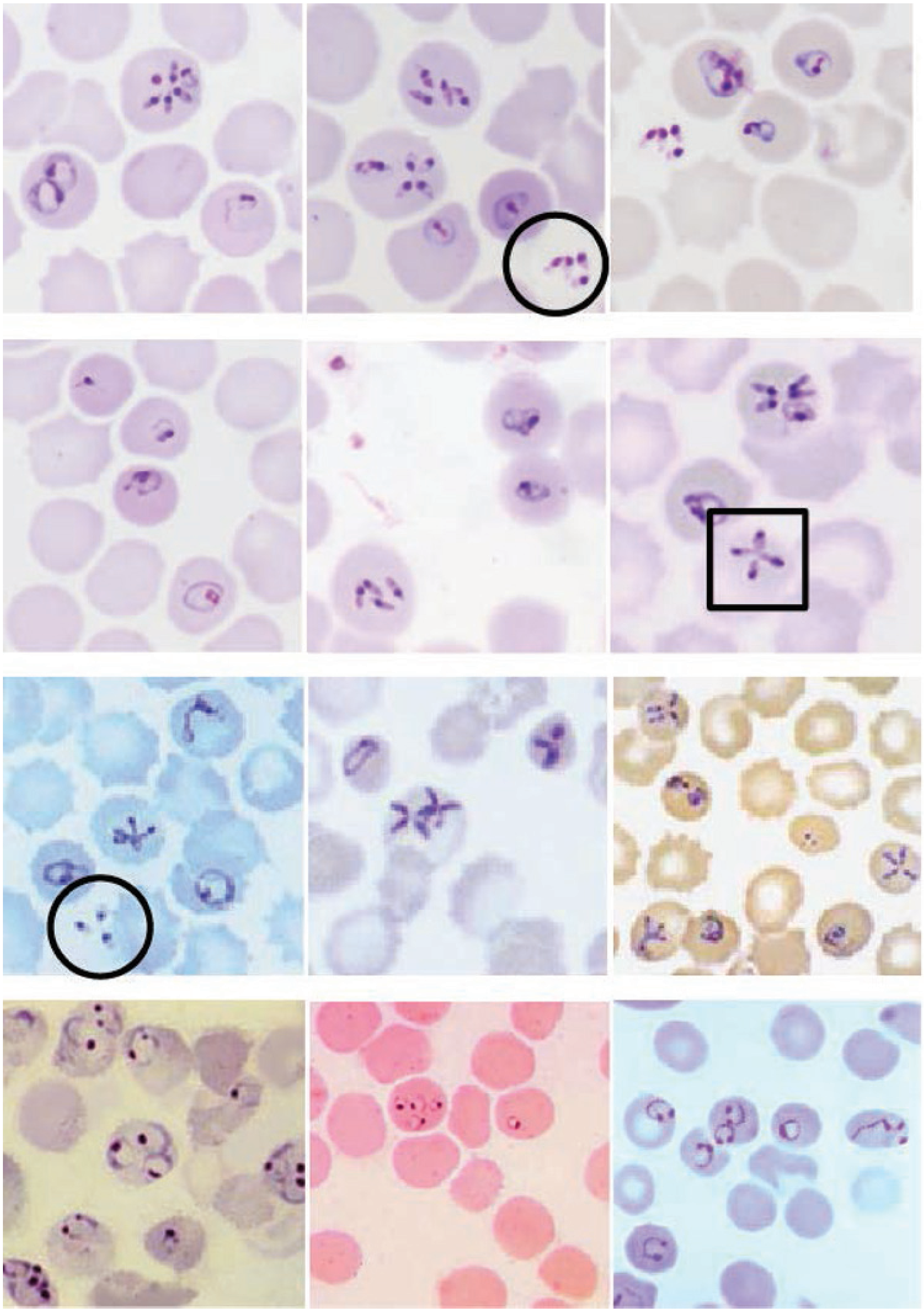


Figure 26.23 (Top three rows) Nine examples of blood films containing the ring-like forms of *Babesia* spp. Shown are various ring forms, multiple rings per cell, some rings present outside of the red blood cells (circles), and the typical image of the Maltese cross configuration of the four rings (square). (Bottom) *Plasmodium falciparum* rings for comparison. Note: it is rare to see *Plasmodium* rings outside of the RBC.
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Onset of serious illness is sudden, with hemoglobinuria as the most common sign. Clinical signs of *B. divergens* infection are easily confused with those of malaria including jaundice, nonperiodic fever, sweats, shaking, headache, vomiting, and diarrhea. Most cases of *B. divergens* infection result in major organ failure and death within 4 to 7 days after the onset of hemoglobinuria. However, prompt therapy with blood transfusion and respiratory support has reduced the mortality rate.

Diagnosis

Babesiosis needs to be diagnosed for various reasons: (i) without treatment, babesiosis can lead to severe illness; (ii) the disease can persist for a long period as an asymptomatic infection, which could lead to posttransfusion cases; and (iii) effective specific treatments are available. Most documented human patients have had a low parasitemia; thus, both thick and thin blood films stained with one of the blood stains must be examined. Although this strategy is rarely used, when the organisms cannot be demonstrated in a suspected patient, blood can be inoculated intraperitoneally into a hamster or gerbil. The organisms can usually be demonstrated within a few days, with the same morphology as that seen in humans.

Conventional Microscopy. Often, when the diagnosis of babesiosis is considered, only a single blood specimen is submitted to the laboratory for examination; however, single films or specimens cannot be relied on to exclude the diagnosis, especially with a low parasitemia.

It is recommended that both thick and thin blood films be prepared on admission of the patient, and at least 200 to 300 oil immersion fields should be examined on both films before a negative report is issued (20, 21). Since one set of negative films will not rule out babesiosis, additional blood specimens should be examined over a 36-h time frame. Although Giemsa stain has been used for all parasitic blood work, the organisms can also be seen if other blood stains, such as Wright's stain or one of the rapid stains, are used. If the WBC morphology is acceptable, then any parasites present will also exhibit typical morphology. Blood collected with the use of EDTA anticoagulant is acceptable; however, it is important to remember that the proper ratio between blood and anticoagulant is necessary for good organism morphology. Heparin can also be used, but EDTA is preferred. Finger stick blood is also acceptable. The blood should be free flowing when taken for smear preparation and should not be contaminated with alcohol used to clean the finger prior to the stick.

Automated Hematology Analyzer. Potential diagnostic problems with the use of automated differential instruments have been reported. Cases of babesiosis and malaria have been missed when blood smears were examined by these methods. The number of fields scanned by a technologist on instrument-read smears is quite small; therefore, a light parasitemia will almost surely be missed. Failure to make the diagnosis results in a delay of a number of days before therapy is instituted. Although the majority of these instruments are not designed to detect intracellular blood parasites, routine use of the automated systems may pose serious diagnostic problems, particularly if the suspected diagnosis is not conveyed to the laboratory.

Alternative Methods. Stained thick and thin blood films have been used to diagnose babesiosis for years and have always been considered the "gold standard." However, a number of alternative methods have been developed, including different approaches to microscopy, flow cytometry, biochemical methods, immunoassay, and molecular methods.

Microhematocrit centrifugation. Microhematocrit centrifugation, using the QBC tube (glass capillary tube and closely fitting plastic insert [QBC malaria blood tubes; Becton Dickinson, Sparks, MD]), has been used for the detection of blood parasites. At the end of centrifugation of 50 to 110 µl of capillary or venous blood (5 min in a QBC centrifuge [14,387 × *g*]), parasites or RBCs containing parasites are concentrated into a small, 1- to 2-mm region near the top of the RBC column and are held close to the wall of the tube by the plastic float, thereby making them readily visible by microscopy. Tubes precoated with acridine orange provide a stain that induces fluorescence in the parasites. This method automatically prepares a concentrated smear that represents the distance between the float and the walls of the tube. The tube is placed into a plastic holder, a drop of oil is applied to the top of the hematocrit tube, a coverslip is added, and the tube is examined with a 40× to 60× oil immersion objective (the objective must have a working distance of 0.3 mm or greater).

Note Although a *Babesia* sp. infection can be detected by the microhematocrit centrifugation method (which is more sensitive than use of the thick or thin blood smear), *appropriate thick and thin blood films must be examined to accurately confirm babesiosis compared with malaria.*

Molecular methods. Certainly PCR has been found to be more sensitive than blood film examination that often results in false-negative results due to low blood parasitemias. One real-time PCR assay was shown to be 100% specific when tested against a panel of 24 organisms consisting of *Babesia microti*, other *Babesia* species, *Plasmodium* species, tick-borne and other pathogenic bacteria, and other blood-borne parasites. The results using clinical specimens demonstrate that the assay can detect infections of lower parasitemia than can be detected by microscopic examination (73).

Serologic methods. Serologic studies have been reported; however, cross-reactivity has been seen among different species of *Babesia* and several species of *Plasmodium*. False-positive results have been seen in patients with connective tissue disorders such as systemic lupus erythematosus and rheumatoid arthritis. Titers of 1:128 to 1:256 rarely represent false positives; however, blood donor screening at a 1:64 titer may result in occasional false positives. False-negative results can be seen in immunosuppressed patients or in patients from whom blood specimens are drawn early in the course of the infection. Use of the indirect fluorescent-antibody test is helpful in the diagnosis of *B. microti* infections, particularly chronic infections. This test is specific and sensitive and is the currently recommended serologic method. Antibody can usually be detected when patients are first diagnosed; titers can remain elevated for 13 months to 6 years.

B. microti, the most common cause of transfusion-transmitted babesiosis, is widely endemic in the northeastern and upper midwestern United States. High seroprevalence in endemic areas limits antibody-based donor screening. A high-performance molecular test is needed to identify donors in the preseroconversion window phase as well as to discriminate past serologic exposure with parasite clearance from continued parasitemia. A highly sensitive and specific, quantitative real-time PCR-based assay for detection of *B. microti* has been developed that could have a useful role in blood screening (74).

KEY POINTS—LABORATORY DIAGNOSIS

Babesiosis

1. Blood films should be prepared on admission of the patient. Travel history and/or possible exposure to tick-infested areas, recent blood transfusion, or splenectomy information is very important.
2. Both thick and thin blood films should be prepared. At least 200 to 300 oil immersion fields should be examined before the smears are considered negative.
3. Wright's, Wright-Giemsa, Giemsa, or rapid stain can be used; if the WBC morphology is acceptable, any parasites would also exhibit typical morphology. Color variation is not critical.
4. *Babesia* parasites may be missed by automated differential instruments. Even with technologist review of the smears, a light parasitemia is very likely to be missed.
5. There does not have to be any significant history of travel outside the United States. If organisms are seen, they tend to mimic *P. falciparum* rings.
6. No morphological stages other than ring forms will be seen. The classic arrangement of the four rings (Maltese cross) is not always seen.
7. *One negative set of blood smears does not rule out a* Babesia *infection.*
8. The quantitative buffy coat system can also be used and has shown 100% correlation with Giemsa-stained peripheral blood smears for the detection of parasitemia in patients with babesiosis. However, confirmation of morphologic differences between *Babesia* and malarial rings might require examination of Giemsa-stained blood films.
9. The indirect fluorescent-antibody test and possibly PCR are recommended. Hamster inoculation is helpful but is neither realistic nor practical for most diagnostic laboratories.

Treatment

Often, babesiosis can be effectively managed with supportive care. Currently, the combination of atovaquone plus azithromycin has been recommended as the standard regimen for human babesial infection. Clindamycin plus quinine is recommended for severe symptoms with *B. microti* infection and for all *B. divergens* infections. Clindamycin plus quinine is also the treatment of choice for infections caused by *B. duncani*, *B. divergens*-like organisms, or *B. venatorum*. Exchange transfusion should be considered for severely ill patients and those with high (>10%) parasitemia or pulmonary, renal, or hepatic compromise when infection is caused by *B. microti*, and is recommended for cases of *B. divergens* infection. Highly immunosuppressed patients should be treated for a minimum of 6 weeks and at least 2 weeks past the last positive smear. High doses of azithromycin (600 to 1,000 mg) have been used in combination with atovaquone for the treatment of immunocompromised patients. Resistance to atovaquone plus azithromycin has been reported in immunocompromised patients treated with a single subcurative course of this regimen.

Any person with babesiosis acquired in Europe should be treated on an emergency basis. Such patients should receive prompt specific therapy to reduce parasitemia and to prevent the extensive hemolysis and renal failure that may follow. The element of time becomes critical; with the onset of hemoglobinuria, rapidly increasing intravascular hemolysis leads to renal failure. Treatment for these seriously ill patients can include exchange transfusion, intravenous clindamycin, and oral quinine.

Epidemiology and Prevention

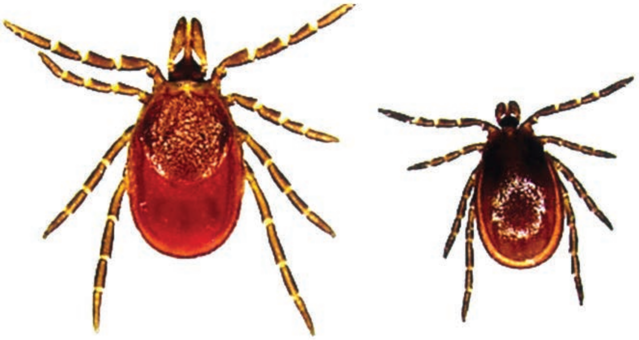
The public health notice that was distributed to the Nantucket Board of Health indicated that *Babesia* parasites are transmitted from mice to humans primarily by the bite of the deer tick *Ixodes scapularis* and that individuals with the infection may be asymptomatic. The notice indicated that prevention still relies on the avoidance of ticks or their prompt removal once detected, since there are apparently no fully effective tick repellents. If symptoms appear 1 to 2 weeks after a tick bite, a physician should be consulted. In a study of Block Island, RI, from 1991 through 2000, the incidence of babesial infection appeared to be at least 10-fold greater than previously recognized elsewhere.

Another problem is related to blood donors. The duration of parasitemia following asymptomatic or symptomatic infection is not known. In areas where the infection is endemic, exclusion of blood donors who are febrile, who are known to have had babesiosis, or who have an anti-*Babesia* titer of 116 or higher may reduce the risk. However, until a practical, effective, and sensitive screening method is developed, cases of transfusion-induced babesiosis will probably continue to occur.

It is now well established that under blood-banking conditions (4°C for 30 days), *B. microti* can remain infective and that transfusion-acquired infection with this parasite could occur during the normal storage time for blood.

There is no vaccine for human babesiosis. The most common and accepted approach used to protect humans from infection involves methods to reduce the tick density (Fig. 26.24 and 26.25). A number of methods have been used, including spraying acaricidal emulsions on vegetation. Repellents are useful for personal protection; however, daily examination of the body surface of a person who may have come in contact with ticks is critical. If ticks are found, they should be removed immediately to take advantage of the time lag between attachment to the human and transmission of infection (usually 50 to 60 h).

Figure 26.24 *Ixodes scapularis* (blacklegged or deer tick), which transmits *Babesia* sp. (Left) Adult female; (right) adult male. (Courtesy of the Department of Entomology, University of Wisconsin-Madison, Heriberto Verdugo M. and Darby S. Murphy; http://labs.russell.wisc.edu/wisconsin-ticks/wisconsin-ticks/ixodes-scapularis-black-legged-ticks/pictures-of-deer-ticks-and-other-ticks.)
doi:10.1128/9781555819002.ch26.f24



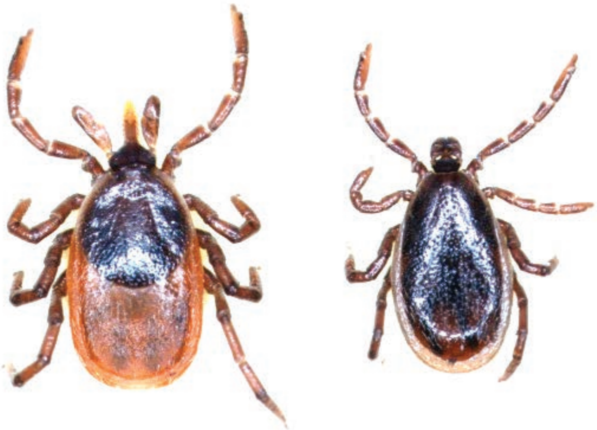


Figure 26.25 *Ixodes scapularis* ticks. (Left) Female; (right) male. (Courtesy of Oklahoma State University, http://entoweb.okstate.edu/ddd/insects/blackleggedtick.htm.)
doi:10.1128/9781555819002.ch26.f25

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27

Leishmaniasis

Cutaneous Leishmaniasis: General Comments

Old World (Eastern) Leishmaniasis: Cutaneous Leishmaniasis

New World (Western) Leishmaniasis: Cutaneous Leishmaniasis

Visceral Leishmaniasis: General Comments

Old World (Eastern) Leishmaniasis: Visceral Leishmaniasis

New World (Western) Leishmaniasis: Visceral Leishmaniasis

Leishmania spp. are protozoa belonging to the family *Trypanosomatidae* (Table 27.1). They are obligate intracellular parasites that are transmitted to the mammalian host by the bites of infected sand flies. On the basis of development in sand flies, the genus *Leishmania* has been divided into two subgenera. In the subgenus *Leishmania*, the development of organisms is restricted to the anterior portion of the alimentary tract of the sand fly, while in the subgenus *Viannia*, the parasites develop in the midgut and hindgut of the sand fly.

Leishmaniasis is mainly a zoonosis, although in certain areas of the world there is primarily human-vector-human transmission. The World Health Organization estimates that 1.5 million cases of cutaneous leishmaniasis (CL) and 500,000 cases of visceral leishmaniasis (VL) occur every year in 88 countries. Estimates indicate that there are approximately 350 million people at risk for acquiring leishmaniasis, with 12 million currently infected (1–3).

Leishmaniasis refers to a diverse group of diseases, with the spectrum of disease depending on the infecting species. Disease syndromes range from self-healing cutaneous lesions to debilitating mucocutaneous infections, subclinical viscerotropic dissemination, and fatal visceral involvement. Since disease presentations vary considerably, a well-defined classification system based on clinical findings is difficult and sometimes confusing (4–13). With recent outbreaks in many areas of the world, including Brazil, India, Italy, Spain, Sudan, and Kenya, leishmaniasis has become more widely recognized as an important emerging infectious disease in many developed as well as underdeveloped countries.

Cutaneous Leishmaniasis. The most common presentation is localized CL, most frequently caused by *Leishmania major* and *Leishmania tropica* in the Old World and *Leishmania braziliensis*, *Leishmania mexicana*, and related species in the New World. Spontaneous healing is the rule, but requires months to years and varies by species.

Mucosal/Mucocutaneous Leishmaniasis. Mucosal leishmaniasis (ML) usually occurs months or years after healing of primary CL, most commonly due to *L. braziliensis*, and can cause destruction of the nasal septum, palate, and other

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TABLE 27.1 Taxonomic classification of *Leishmania* parasites pathogenic for humans[a]

Taxon	Old World[b]				New World[c]			
Family	*Trypanosomatidae*							
Genus	*Leishmania*							
Subgenus	*Leishmania*				*Leishmania*		*Viannia*	
Complex	*L. major*	*L. tropica*	*L. aethiopica*	*L. donovani*	*L. donovani*	*L. mexicana*	*L. braziliensis*	*L. guyanensis*
Species	*L. major*	*L. tropica*	*L. aethiopica*	*L. donovani* *L. infantum*	*L. chagasi*	*L. mexicana* *L. venezuelensis* *L. garnhami* *L. amazonensis* *L. pifanoi*	*L. braziliensis* *L. peruviana* *L. colombiensis* *L. lainsoni* *L. shawi* *L. naiffi*	*L. guyanensis* *L. panamensis*
Hybrid species[d]							*L. braziliensis/L. panamensis* *L. braziliensis/L. guyanensis*	

[a] The classification scheme is based on biochemical and molecular characteristics.

[b] Animal parasites of the Old World (*L. arabica*, *L. turanica*, and *L. gerbilli*) are not listed.

[c] Animal parasites of the New World (*L. aristedesi*, *L. enrietti*, *L. forattinii*, *L. deanei*, *L. equatorensis*, and *L. bertigi*) are not shown; although human infection with these species has not yet been identified, there is often a time lag between the discovery of new animal genera and confirmation of human infection. In many cases, the relevant vectors have been found to bite humans as well as the normal animal hosts.

[d] Strains with phenotypic and genotypic characters of two species have been recorded in different geographic areas of Latin America; either they represent strains that originated from a common ancestor or they are the result of genetic exchange.

mucosal structures, leading to devastating facial mutilation and, rarely, death from airway involvement. Other forms include disseminated cutaneous leishmaniasis (DCL), which is a diffuse, nodular, nonulcerating disease, and leishmaniasis recidivans, which produces localized, slowly progressive, nonhealing lesions. Both are rare and difficult to treat and can be severe.

Visceral Leishmaniasis. VL (kala azar) is usually caused by *Leishmania donovani* and *Leishmania infantum* and is characterized by progressive fever, weight loss, splenomegaly, hepatomegaly, hypergammaglobulinemia, and pancytopenia. Kala azar is lethal in nearly all untreated cases, and in treated patients, case-fatality rates are often 10% or higher.

Post-Kala Azar Dermal Leishmaniasis. Post-kala azar dermal leishmaniasis (PKDL) is a chronic rash seen in apparently cured kala azar patients in South Asia and the Horn of Africa. PKDL patients present with erythematous or hypopigmented macules, sometimes progressing to plaques or nodules. In Sudan, PKDL is reported to resolve without treatment in most mild cases, while the condition is said to require universal treatment in South Asia. A high percentage of patients with kala azar develop PKDL in Sudan, with lower percentages in South Asia.

Leishmaniasis in the United States. Cases of leishmaniasis are diagnosed each year in the United States and can be attributed to immigrants from countries with endemic infection, military personnel, and American travelers. Another concern is the potential for more infections occurring in areas of endemic infection in Texas and Arizona (14–19). Organisms can remain latent for years, and even when the potential exposure history is in the past, leishmaniasis should be considered, particularly in immunocompromised patients such as those infected with HIV (20–25).

The taxonomy of the *Leishmania* spp. is controversial and reflects the development of multiple parameters on which classification is based. Previously, organisms were classified based on the clinical disease picture and geographic locations; with the development of criteria based on biochemical and genetic data, the taxonomic groupings no longer coincide with recognized disease entities.

Cutaneous Leishmaniasis: General Comments

There are multiple clinical presentations associated with cutaneous leishmaniasis; however, five different syndromes are currently recognized: (i) localized ulcerative or nodular cutaneous leishmaniasis (CL), (ii) mucosal leishmaniasis (ML), (iii) leishmaniasis recidivans (LR); (iv) diffuse or disseminated cutaneous leishmaniasis (DCL); and (v) post-kala azar dermal leishmaniasis (PKDL). Immunocompromised patients may have atypical presentations (1).

Old World (Eastern). LR is most common in the Middle East. PKDL is seen in India and East Africa (Kenya and the Sudan) following treatment of kala azar with pentavalent antimony.

New World (Western). CL is found in the Americas from southern Texas to northern Argentina. ML is most common in the jungles of Amazonia (especially in southern Brazil), but cases have been reported throughout the Americas. Most DCL infections are seen in Brazil, the Dominican Republic, Ethiopia and Venezuela, but there are sporadic infections in Bolivia, Colombia, Honduras, Mexico, Peru, and the southern United States.

Old World (Eastern) Leishmaniasis: Cutaneous Leishmaniasis

Leishmania major, *L. tropica*, *L. aethiopica*, and, rarely, *L. infantum* cause cutaneous disease in the Old World; disease manifestations include nodular and ulcerative skin lesions (Table 27.2; Fig. 27.1). Local geographic names for CL include Oriental sore, Baghdad boil, Delhi boil, Biskra button, and Aleppo evil. Although Wright was credited with the first detailed description of the cutaneous form of the disease, it had already been described by Borovsky in 1898 in an obscure military medical journal.

Life Cycle and Morphology

The life cycle can be seen in Fig. 27.2. The parasite is found in two morphologic forms in the life cycle, the amastigotes and the promastigotes. The amastigotes are small (3 to 5 μm in diameter), ovoid, nonmotile intracellular forms, while the promastigotes are elongated, motile, extracellular stages (1.5 to 3.5 by 15 to 20 μm with a single free flagellum 15 to 28 μm long) (Fig. 27.3 and 27.4). The form introduced into the skin of the mammalian host by the sand fly is the promastigote. The organism is engulfed by reticuloendothelial (RE) cells of the mammalian host, where the parasite transforms into the intracellular amastigote form (Leishman-Donovan body). Apparently, this process takes approximately 12 to 24 h. The large nucleus and small kinetoplast in the amastigotes can be seen in tissue after staining with Giemsa, Wright's, or other blood stains. The short intracytoplasmic portion of the flagellum can also be seen within some of the amastigotes. The amastigotes multiply by binary fission within the macrophage until the cell is destroyed, and the released parasites are phagocytized by other RE cells or ingested by sand flies. This phase is chronic and can last from months to years, even a lifetime, depending on the species involved. In CL, the amastigotes remain confined to the skin, usually as skin lesions such as raised papules or ulcers. Lesions usually occur on exposed parts of the body such as the face, hands, feet, arms, and legs; uncommon sites include the ears, tongue, and eyelids.

During a blood meal taken by the sand fly (genus *Phlebotomus*), amastigotes are ingested and transform into the promastigote stage. Promastigotes multiply by longitudinal fission in the insect gut. Stages found in the sand fly vary from rounded or stumpy forms to elongated, highly motile

TABLE 27.2 Old World cutaneous leishmaniasis, subgenus *Leishmania*

Species and sand fly vector	Human disease and lesion	Geographic distribution	Important hosts	Comments
L. major: *Phlebotomus papatasi*, *P. duboscqi*, *P. salehi*	Cutaneous leishmaniasis (Oriental sore; rural, zoonotic); lesions may number >100; wet lesion with crusting; necrotic and exudative	North Africa, sub-Saharan Africa, Central and West Asia	Great gerbil (*Rhombomys opimus*), fat sand rat (*Psammomys obesus*)	Primarily an infection of desert rodents; gerbil infection rate may reach 30%; village inhabitants near gerbil burrows may have infection prevalence rates of 100%; major problem for rural settlers in Israel, military personnel in the Sinai Desert, Israel, Iraq, and Iran
L. tropica: *Phlebotomus sergenti*, *P. papatasi*, *P. chaudaudi*	Cutaneous leishmaniasis (Oriental sore; urban, anthroponotic); lesions usually ~2; lesions more swollen, less necrotic; less exudate than with *L. major*; leishmaniasis recidivans also seen (chronic lesions on face or other exposed areas, persist for years)	Middle East, Mediterranean littoral, India, Pakistan, Central Asia, Iran, Iraq	Dogs and humans	Major epidemic of anthroponotic CL has occurred in war-ravaged Kabul, Afghanistan, with thousands of cases identified; responsible for viscerotropic syndrome in a small group of American military personnel infected in the Persian Gulf War; extensive scarring can lead to disfigurement; can last for up to 40 years
L. aethiopica: *Phlebotomus longipes*, *P. pedifer*	Cutaneous leishmaniasis, diffuse cutaneous leishmaniasis; lesions more swollen, less necrotic; barely exudative	Ethiopia, Kenya, bordering countries	Rock hyraxes (*Heterohyrax brucei*, *Procavia* spp.)	Human disease usually self-healing, small number develop nonhealing disseminated CL

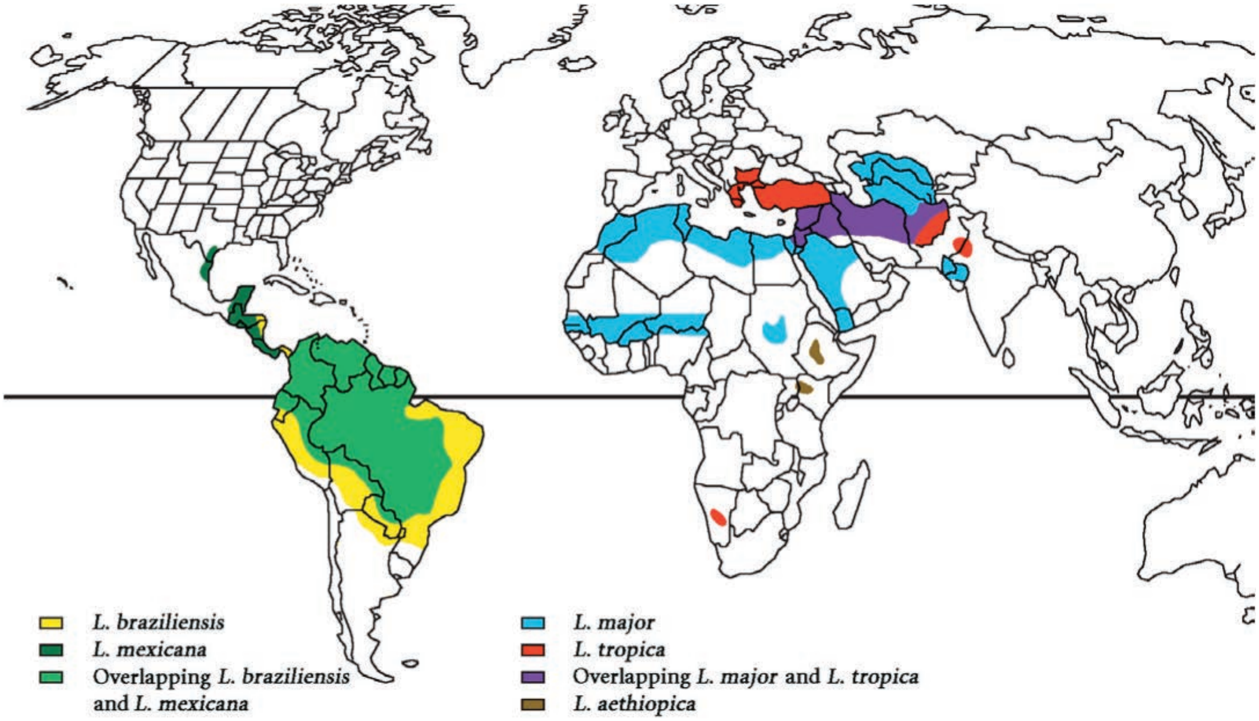

L. braziliensis
L. mexicana
Overlapping L. braziliensis and L. mexicana
L. major
L. tropica
Overlapping L. major and L. tropica
L. aethiopica

Figure 27.1 Geographic distribution of the agents of cutaneous leishmaniasis (courtesy of reference 1). doi:10.1128/9781555819002.ch27.f1

metacyclic promastigotes. The metacyclic promastigotes migrate to the hypostome of the sand fly, where they are inoculated into humans when the sand fly takes its next blood meal. Various surface molecules on the promastigote, such as gp63 and lipophosphoglycans, help bind the parasite to the host macrophage receptors, thus allowing it to be phagocytized. Depending on the species, the duration of the life cycle in the sand fly varies from 4 to 18 days.

Sand flies are pool feeders and possess cutting mouthparts that slice into the skin, allowing a small pool of blood to form. Thus, although they are small flies (2 to 3 mm), the bite can be painful. In most foci of endemicity, the vast majority of sand flies are uninfected; however, those that are infected are very efficient vectors. Infected flies probably remain so for life, although this is just a matter of weeks. Under optimal conditions, transformation of the amastigotes to promastigotes generally takes 5 to 7 days; by that time, the female phlebotomine fly is ready to take her next blood meal.

Leishmaniavirus is a double-stranded RNA virus that persistently infects some strains of *Leishmania*. There is a great deal of interest in the possibility that the presence of

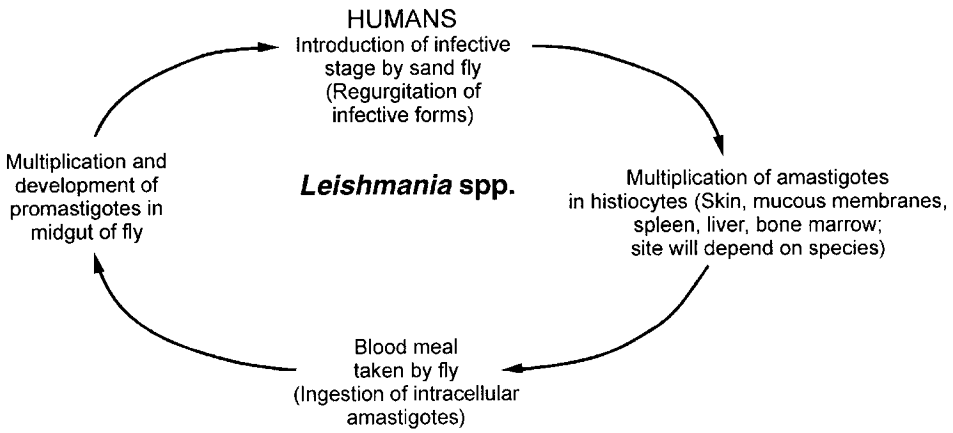

HUMANS
Introduction of infective stage by sand fly (Regurgitation of infective forms)
Multiplication of amastigotes in histiocytes (Skin, mucous membranes, spleen, liver, bone marrow; site will depend on species)
Blood meal taken by fly (Ingestion of intracellular amastigotes)
Multiplication and development of promastigotes in midgut of fly
Leishmania spp.

Figure 27.2 *Leishmania* life cycle. doi:10.1128/9781555819002.ch27.f2

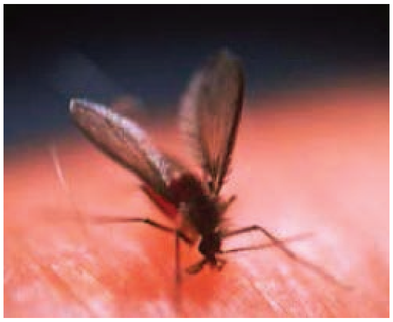


Figure 27.3 Sandfly vector of leishmaniasis taking a blood meal through human skin; note the "V" position of wings over the thorax, erythema around bite site, and hairy body and wings (courtesy of WHO, S. Stammers).
doi:10.1128/9781555819002.ch27.f3

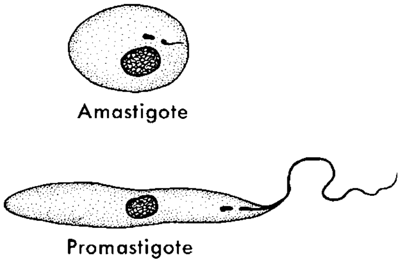
this virus may be able to alter the parasite phenotype and may affect disease pathogenesis. The virus has been detected in cultured parasites and has also been detected in human biopsy samples prior to manipulation in culture (26).

Clinical Disease

The incubation period of CL is usually 2 to 8 weeks but can be as long as several years. The first sign of cutaneous disease is a lesion (generally a firm, painless papule) at the bite site. Although a single lesion may appear insignificant, multiple lesions of *L. major* or disfiguring facial lesions of *L. tropica* may be psychologically or physically devastating. In general, all lesions on a patient have a similar appearance and progress at the same speed.

The original lesion may remain as a flattened plaque or may progress, with the surface becoming covered with fine, papery scales. These scales are dry at first but later become moist and adherent, covering a shallow ulcer. As the ulcer enlarges, it produces exudate and may develop a crust. The edge of the lesion is usually raised. Depending on the species, satellite lesions are common and merge with the original lesion (Fig. 27.5).

Figure 27.4 *Leishmania* stages found in human and insect hosts. (Illustration by Nobuko Kitamura.)
doi:10.1128/9781555819002.ch27.f4



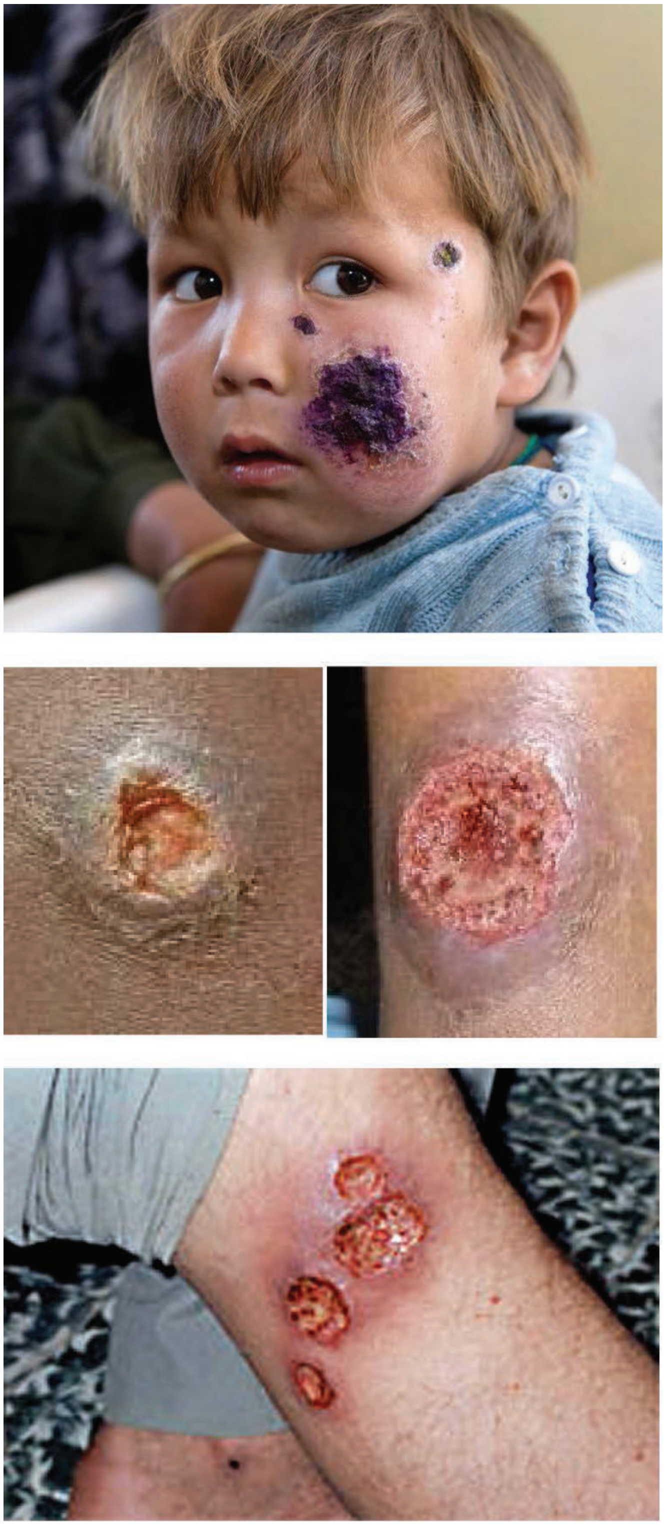


Figure 27.5 (Top) Child with cutaneous leishmaniasis (CL) awaiting treatment in Kabul, Afghanistan (courtesy of WHO, C. Black). Kabul, Afghanistan harbors the largest number of CL patients in the world. (Middle and bottom) CL lesions.
doi:10.1128/9781555819002.ch27.f5

***L. major* (Rural Disease).** *L. major* causes rural disease, a variety of CL. Multiple lesions are present and are accompanied by marked inflammation and crusting. The center of the lesion tends to be necrotic and exudative, forming a loose crust. The lesions tend to mature and heal relatively quickly, often lasting only a few months. Lymphatic spread may occur in *L. major* infections, with subcutaneous nodules in a linear distribution and regional lymphadenopathy; if the initial lesion is on the hand, this clinical presentation may resemble sporotrichosis. *L. major* infections may result in severe scarring.

From August 2002 to February 2004, over 500 cases of parasitologically confirmed cases of CL were found in U.S. military personnel serving in Afghanistan, Iraq, and Kuwait. Currently, the number of cases may be 1,500 or more; many feel this number may be underestimated due to cases that have been misdiagnosed. The majority of these cases were probably acquired in Iraq, with *L. major* being confirmed for most of the cases through isoenzyme electrophoresis of cultured parasites (6, 27). Based on the data from Fort Campbell, KY, approximately 1% of troops returning from Iraq were diagnosed with CL, most by laboratory confirmation, including PCR.

***L. tropica* (Urban Disease).** The lesions of urban disease (a type of CL) caused by *L. tropica* occur singly or two at a time, develop more slowly than those caused by *L. major*, and can take a year or more to heal. They tend to be more swollen and less necrotic, with less exudate and a thicker crust.

***L. tropica* (Leishmaniasis Recidivans).** LR is generally associated with *L. tropica*. Small, nonulcerating lesions begin to appear, mainly on the margins of healed lesions, and continue to expand the limits of the original scar (Fig. 27.6). Disfigurement due to extensive scarring can develop. This condition is thought to be associated with a strong cell-mediated immune response and tends to occur primarily in Iran and Iraq. Although there is a strong immune response on the part of the host, not all amastigotes are eliminated, and the infection can last for up to 40 years. The patient's physical appearance, as well as histologic sections, resemble those of cutaneous tuberculosis; therefore, the name "lupoid leishmaniasis" has also been used. Parasites may be very difficult to demonstrate; however, culture or animal inoculation has been successful. The leishmanin skin test (LST) (Montenegro test) is strongly positive in these patients.

***L. aethiopica* (Cutaneous Leishmaniasis).** Lesions of CL caused by *L. aethiopica* are the least inflamed but are more swollen. There may be very little exudate, but scaling and exfoliation of the dermis occur. These lesions tend to remain for years prior to complete healing.

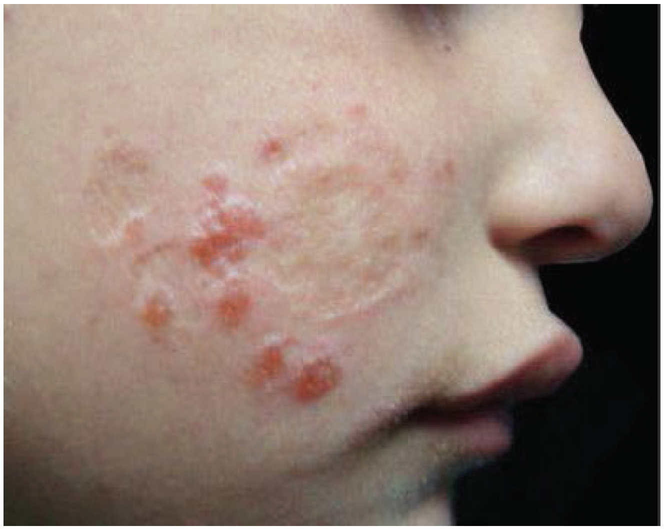


Figure 27.6 Leishmaniasis recidivans (LR) is a rare complicating form of cutaneous leishmaniasis caused by *L. tropica*. After healing of the initial lesion, a few soft red papules may appear at or around the scar of the previously healed lesion that spread peripherally with no tendency for spontaneous healing. Few parasites are found in the skin smear or biopsy. (Courtesy of the Global Skin Atlas, submitted by Nameer Al-Sudany; http://www.globalskinatlas.com/imagedetail.cfm?TopLevelid=2268&ImageID=5064&did=458.)
doi:10.1128/9781555819002.ch27.f6

***L. aethiopica* (Diffuse Cutaneous Leishmaniasis).** Diffuse or disseminated CL (DCL) is an anergic form of CL in which neither humoral nor cell-mediated immune responses are functional; however, delayed hypersensitivity to tuberculin appears to be normal. DCL is caused by *L. aethiopica* and is seen in Ethiopia and Kenya; approximately 100 cases have been reported. The parasites are found in the skin, and the disease is characterized by the formation of thickened skin in plaques, papules, or multiple nodules. This condition is similar to that associated with the specific anergy in lepromatous leprosy, and DCL was originally confused with and thought to be leprosy. Although lesions are usually seen on the face, nose, limbs, and buttocks, they can occur over the entire body (Fig. 27.7). The nodules are often described as soft and fleshy, while those of leprosy are generally more indurated. Although organisms have rarely been recovered from blood or bone marrow, there are no visceral lesions associated with this form of leishmaniasis. Treatment is difficult, and the infection may last for the life of the patient. The LST remains negative unless treatment is effective and recovery occurs.

Diagnosis

In areas of the world where physicians are very familiar with leishmaniasis, the diagnosis may be made on clinical

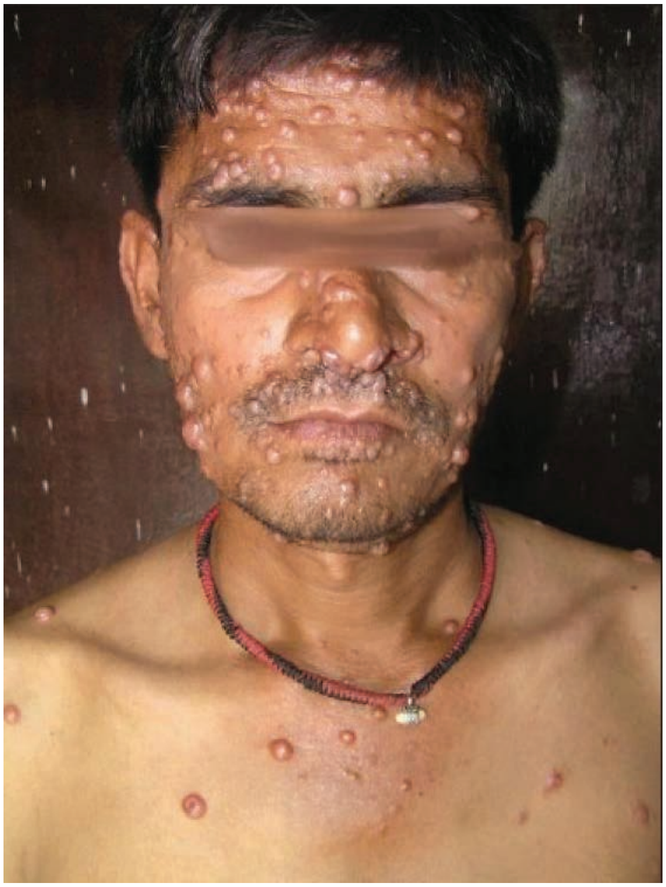


Figure 27.7 Diffuse cutaneous leishmaniasis seen in an HIV-positive patient (courtesy of Purohit HM et al, *Indian J Sex Transm Dis* **33:**62–64, 2012).
doi:10.1128/9781555819002.ch27.f7

grounds. However, in other areas of the world, where the disease is rare, the condition may not be recognized. Definitive diagnosis depends on demonstrating the amastigotes in tissue specimens or the promastigotes in culture (Table 27.3). CL may have to be differentiated from a number of other lesions and diseases, including basal cell carcinoma, tuberculosis, various mycoses, keloid scarring, and lepromatous leprosy.

Specimen Collection. CL normally presents with no visceral involvement or systemic disease. The ability to detect parasites in aspirates, scrapings, or biopsy specimens depends on the number of amastigotes present, the level of the host immune response, the absence or presence of bacterial and/or fungal contamination within the lesion, and whether the specimen is collected from an active or healing lesion. If the patient has multiple lesions, specimens should be collected from the more recent or active lesions. These lesions should be thoroughly cleaned with 70% alcohol, and necrotic debris should be removed to prevent the risk of bacterial and/or fungal contamination of the specimen. This is mandatory if cultures are to be used for isolation and growth of the parasites; excess bacterial or fungal contamination prevents development of the promastigote forms. Also, the specimen should be taken from the advancing margin of the lesion; the central portion of the ulcer contains nothing but necrotic debris.

If specimens are collected by aspiration, scraping, or biopsy, local anesthesia may be used to reduce patient discomfort. The specimen of choice would be a collection of several punch biopsy specimens taken from the most active lesion areas. Biopsy specimens can be divided and used for cultures and touch preparations; some material should always be saved and submitted for routine histology. If biopsy specimens need to be divided, material for culture should be obtained by using sterile techniques and instruments. The biopsy specimen can be used to make impression smears (touch preparations); these smears should be prepared after portions of the specimen have been placed in culture media by using a sterile technique. After any excess blood is cleaned off, a horizontal cut is made through the biopsy core and the cut surface is gently touched to glass slides; multiple slides should be prepared. Once material has been set up for culture and touch preparations have been made, the remainder of the tissue can be sent to the pathology laboratory for routine processing. It is important to remember that the amastigotes are more difficult to identify in tissue sections than in touch preparations; they appear smaller and may be cut at different angles.

Another option involving sterile techniques is to make a perpendicular slit in the margin of the lesion. After blood is removed with sterile gauze, the blade can be used to scrape the inner walls of the slit. Material can be inoculated into culture media and can be smeared onto several glass slides; the use of multiple slides is recommended to increase the chances of seeing the parasites. Fine-needle aspiration can also be performed with a sterile syringe containing sterile saline (0.1 ml) and a 26-gauge needle. The needle is inserted into the outer border of the lesion and rotated several times, after which saline is injected and tissue fluid is aspirated back into the needle. Material can be submitted for culture, and/or smears can then be prepared for staining. Additional techniques and specific methods can be found in chapters 6 to 8.

Specimen Staining. All smears to be Giemsa stained should be air dried and fixed with 100% methanol prior to staining. Any of the other blood stains can also be used. The amastigotes are found within the macrophages or close to disrupted cells (Fig. 27.8). Although this intracellular form may mimic other intracellular parasites (*Histoplasma capsulatum* and some of the microsporidia), the presence of the

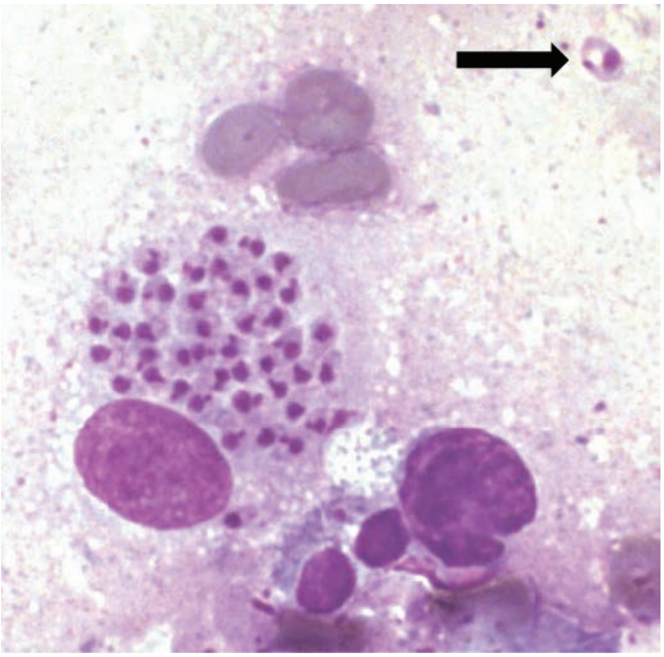
TABLE 27.3 Features of Old World human leishmanial infections related to demonstration of the parasite[a]

Organism	Disease type[b]	Humoral antibodies	Delayed hypersensitivity to leishmanin	Parasite present in clinical specimen	Self-cure	Recommended biopsy specimen
Old World CL						
L. major	CL	Present	Present	Present	Rapid	Skin
L. tropica	CL	Variable	Present	Present	Yes	Skin
	LR	Variable	Strong	Few	Slow	Skin
L. aethiopica	CL	Variable	Weak	Present	Slow	Skin
	DCL	Variable	Absent	Abundant	No	Skin
Old World VL						
L. donovani	VL	Abundant	Absent	Present	Rare	Bone marrow, spleen
	CL (primary lesion)	Variable	Present	Present	Yes	Skin
	PKDL	Variable	Variable	Variable	Variable	Skin
L. infantum	VL	Abundant	Absent	Present	Variable	Bone marrow, spleen

[a] For culture, specimens must be collected aseptically, and in older lesions the parasites may be scant and difficult to recover. Animal isolation involves hamsters and culture media (Novy-MacNeal-Nicolle medium and Schneider's *Drosophila* medium with 30% fetal bovine serum). Serologic testing is most suitable for VL, has little value for CL, and has limited value for MCL. The Montenegro test (LST) is a delayed hypersensitivity reaction to intradermal injection of cultured parasites.

[b] Additional information on relevant diagnostic methods can be found in chapters 7 and 8.

Figure 27.8 Cutaneous leishmaniasis. Smear of skin lesion with amastigotes with the macrophage; Giemsa stain. In these types of preparations, often some of the amastigotes will appear outside of the cells (arrow). (Courtesy of the Armed Forces Institute of Pathology.) doi:10.1128/9781555819002.ch27.f8



intracytoplasmic kinetoplast confirms the identification. After staining, the cytoplasm appears light blue and the nucleus and kinetoplast appear red to purple. In very early and older lesions, very few organisms may be present, so that prolonged examination of the slide may be required.

Culture Media. Media used for the growth of *Leishmania* spp. will also support the growth of bacteria and fungi, hence the need for aseptic techniques in collecting the clinical specimen. If cultures become overgrown with contaminating organisms, the parasites will not survive. It is also important to release the organisms from tissue prior to culture; mincing is recommended. However, tissue grinders are not recommended because of possible damage to the organisms. Culture media that have been used for the recovery and growth of leishmaniae include Novy-MacNeal-Nicolle medium and Schneider's *Drosophila* medium supplemented with 30% fetal bovine serum (see chapter 8). A number of other medium formulations have also been developed and may prove very useful. These include a completely defined medium without serum and macromolecules (28), a semisynthetic fetal calf serum-free liquid medium (29), a new liquid medium with less expensive chemicals (30), and a sensitive new microculture method involving microcapillary tubes (31). Patient cultures should not be set unless the laboratory maintains specific organism strains for quality control checks. Both the control and patient cultures should be examined twice a week for the first 2 weeks and once a week thereafter for up to 4 weeks before they are reported as negative. Promastigote stages

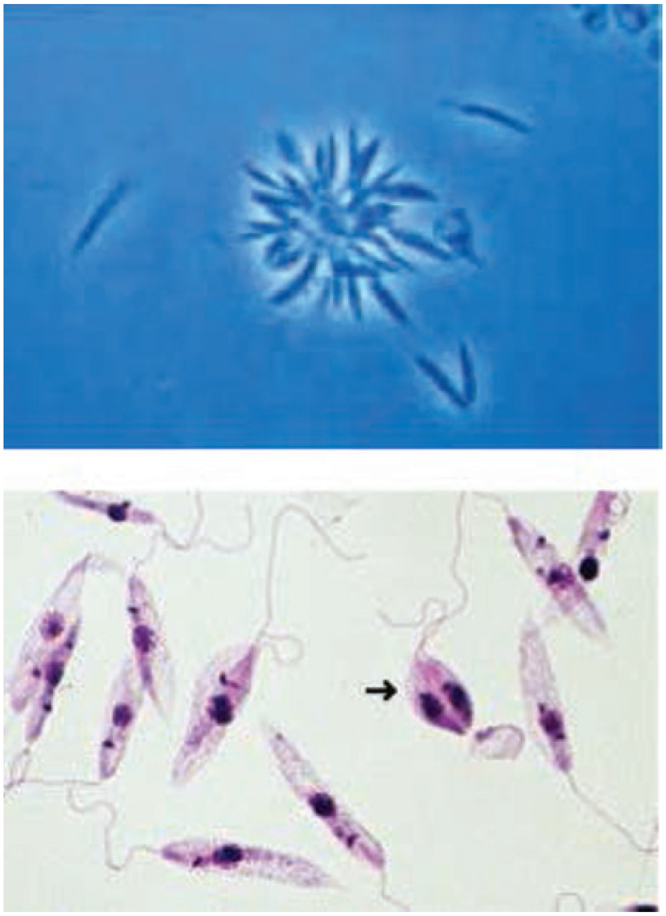


Figure 27.9 *Leishmania* sp. promastigotes in culture. (Upper) Wet mount. (Lower) Stained organisms; note promastigotes dividing by longitudinal binary fission (arrow). (Courtesy of WHO, images from Jorge Alvar.) doi:10.1128/9781555819002.ch27.f9

can be detected microscopically in wet mounts taken from centrifuged culture fluid (Fig. 27.9). This material can also be stained with Giemsa stain to facilitate observation at a higher magnification. A number of molecular methods have been developed for species identification of the promastigotes, including the use of DNA probes, isoenzyme analysis, PCR, and monoclonal antibodies (32–37).

Animal Inoculation. Animal inoculation can be helpful when only a small number of organisms are present; however, most laboratories no longer consider this approach practical. Golden hamsters are inoculated intranasally, and the major argument against this approach is that it may take several months before the animal gives positive responses. A combination of tissue smears, culture, animal inoculation, and histopathology may be required for optimal diagnosis.

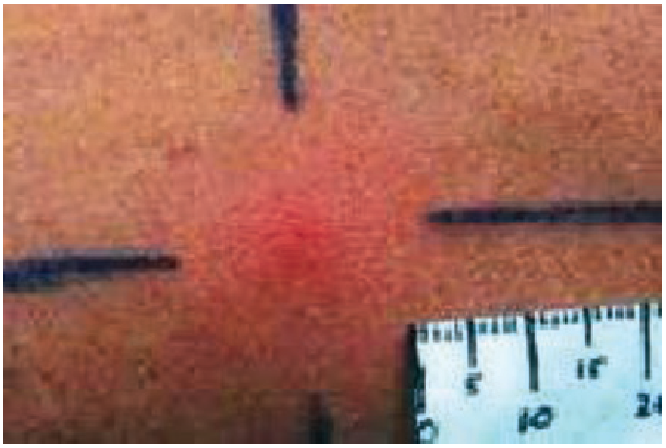


Figure 27.10 LST (Montenegro skin test); positive test result (courtesy of *Dermatology Online Journal*, University of California, Davis, eScholarship http://escholarship.org/uc/item/9ct3h710). doi:10.1128/9781555819002.ch27.f10

Skin Test. The LST (Montenegro test) is useful for epidemiologic population surveys to detect groups at risk for infection. However, the test is not species specific and must be interpreted with caution, particularly since some patients may have more than one type of leishmaniasis. Apparently, cross-reactions do not occur with African or American trypanosomiasis. The LST involves a delayed hypersensitivity reaction to a suspension of killed promastigotes. The reaction is read at 48 and 72 h after injection. Positive reactions are generally seen in patients with cutaneous lesions; however, those with DCL give negative reactions (Fig. 27.10).

Serologic Tests. Although available for epidemiology and/or research studies, serologic tests are not very useful for the diagnosis of CL. Reagent availability is usually limited to areas of endemic infection. Antibody detection is variable in patients with CL; if antibody is present, titers are usually low, and cross-reactions have been seen in patients with leprosy. Patients infected with the viscerotropic form of *L. tropica* seen during Operation Desert Storm have had low or undetectable antibody titers (38).

KEY POINTS—LABORATORY DIAGNOSIS

Old World (Eastern) Leishmaniasis: Cutaneous Leishmaniasis

1. For a complete laboratory diagnosis, microscopic examination of Giemsa-stained touch preparations and cultures is recommended. Other blood stains are also acceptable.
2. Aspirates or biopsy specimens should be taken from the margin or base of the most active lesion (papule or ulcer). The lesion should be cleaned before the sample is collected, to reduce the chances of culture

KEY POINTS—LABORATORY DIAGNOSIS

contamination with fungi or bacteria. Punch biopsies are recommended: one for routine pathology, two for microbiology (squash preps/sterile for culture).

3. Multiple slides should be prepared for examination.
4. Tissue imprints or smears should be stained with Giemsa stain (or one of the stains commonly used for blood smears) (see chapter 7).
5. Amastigote stages should be found within macrophages or close to disrupted cells.
6. If cultures are to be performed, specimens must be taken aseptically and control organism cultures should be set up at the same time (see chapter 8).
7. Cultures should be checked weekly for 4 weeks before they are declared negative.

Treatment

Most Old World CL lesions are self-healing over a period of a few months to several years. This healing process confers immunity to individuals living within the immediate area of endemic infection; therefore, treatment is not recommended unless disfiguring scarring is a possibility. Chemotherapy should be given to patients with lesions on the face, particularly if the lesions are multiple or large. Pentavalent antimonials (sodium stibogluconate [Pentostam] and meglumine antimoniate [Glucantime]) are usually effective but must be given in adequate doses for the complete recommended time frame, often weeks or months (see chapter 36). Miltefosine is another option, with paromomycin or pentamidine being alternatives. Toxicity does not usually present a problem. Specific chemotherapy, including systemic administration, is used much less frequently for Old World leishmaniasis than for the New World disease presentations. The lesions of LR respond to Pentostam at doses used for treatment of VL, and local heat therapy and intralesional injections are commonly used in the Middle East. In treating DCL, pentamidine, given as a single weekly injection for at least 4 months longer than it takes to eliminate parasites from slit skin smears, seems to be a good compromise between efficacy and toxicity. Cutaneous lesions, whether treated or not, should always be cleaned and covered to prevent secondary infections and vector access to the lesion.

In addition to chemotherapy, other more local treatment options have included cryotherapy, heat, and surgical excision of lesions (27). Organisms within the *L. tropica* group do not survive in temperatures above 37°C, and local heat therapy has proven to be effective. The temperature within the lesion should reach 40 to 42°C for 12 h at a time; this can be accomplished by using an infrared heat lamp. The use of hot water in baths or saunas has been helpful in cases of DCL, particularly since the response to chemotherapy is poor.

The administration of Pentostam as direct injections into the lesion has also been used. The dose is divided into four parts and injected into four different quadrants of the lesion (10). Another approach involves the application of a topical formulation of 15% paromomycin–12% methylbenzethonium chloride in soft white paraffin (Pointment or the “El-On” preparation), which decreases the healing time by half. Since this preparation causes multiple local reactions, a number of other preparations have been tried, some effective and some no better than the placebo.

Epidemiology and Prevention

L. major, the cause of rural Oriental sore, is a zoonotic parasite and is found primarily in desert rodents such as gerbils that live in the dry desert areas of central Asia, in southern Russia, throughout Iran and Pakistan, in parts of Iraq, and in northwestern India and China. In areas of Libya, Saudi Arabia, and Israel, the rat is an important host. The infection is maintained in the reservoir population by sand flies that frequent rodent burrows. Human disease is maintained through the vectors *Phlebotomus papatasi* in Sudan and *Phlebotomus duboscqi* elsewhere.

L. tropica, the cause of urban CL, has been isolated from dogs and rats; however, humans are considered to be the primary reservoir and source of infection. Various phlebotomine sand flies (*Phlebotomus sergenti* and *P. papatasi*) transmit the infection, depending on the specific geographic area; areas of endemic infection include southern France, Italy, some Mediterranean islands, the Middle East, Greece, Pakistan, and northwestern India. In the first half of the 20th century, 40 to 70% of Europeans living in Delhi became infected. A major epidemic of CL due to *L. tropica* has occurred in war-ravaged Kabul, Afghanistan, with thousands of cases identified in the displaced population.

L. aethiopica is also a zoonotic parasite, primarily involving the rock hyrax (*Procavia habessinica*) and the tree hyrax (*Heterohyrax brucei*). The organism is found in the Rift Valley in Ethiopia and Kenya. As human habitation moves into deforested mountain areas, exposure to the hyrax becomes much greater and coincides with the vector, *Phlebotomus longipes*, while *Phlebotomus pedifer* is implicated in Kenya.

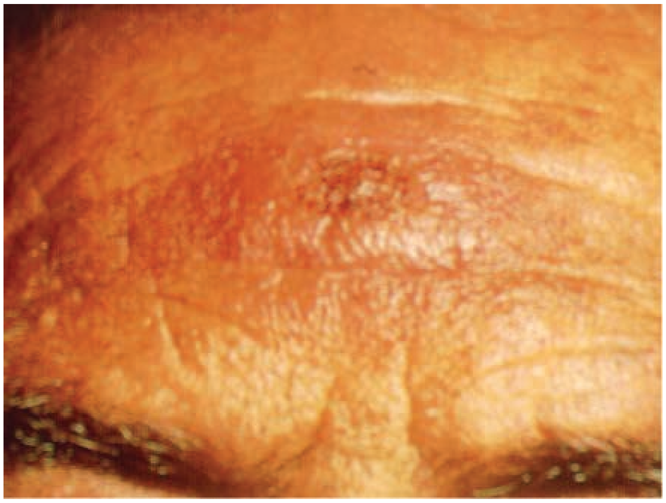
Preventive measures involve vector control; residual spraying with DDT as a means of malaria control was very helpful. However, with the decline and total cessation of spraying in many areas, the leishmaniasis has returned. The infection can also be transmitted by direct contact with infected lesions and mechanically through bites by the stable or dog fly (*Stomoxys* spp.); lesions should be kept covered to prevent this type of transmission by primary or secondary vectors. Application of insect repellents to the skin has also been effective. Although reservoir control has been unsuccessful in most areas, disease incidence has been reduced by poisoning or deep plowing gerbil burrows.

In areas of Iran, Iraq, Israel, Jordan, and Russia where CL is endemic, vaccination has been used for many years. Exudate from a naturally acquired lesion can be inoculated onto an inconspicuous area of the body of a nonimmune person. Another approach is to use live, attenuated *L. major* promastigotes, which are inoculated intracutaneously. The infection is allowed to develop and is usually limited to a single lesion; self-cure proceeds as in a natural infection. The immunized individual will become a carrier and source of infection until the lesion heals; a very small percentage of those vaccinated will develop lesions requiring treatment. Lifelong immunity to the homologous species develops and is comparable to that following a natural infection.

New World (Western) Leishmaniasis: Cutaneous Leishmaniasis

New World CL extends from southern Texas and Arizona to Latin and South America and the Caribbean islands (18). As seen in Old World leishmaniasis, there is a wide range of disease presentation, and separation based on clinical signs and symptoms is not possible (Fig. 27.11 and 27.12). Organisms in this group belong to the subgenera *Leishmania* and *Viannia* (Table 27.1). Within the subgenus *Leishmania* is the *L. mexicana* complex (Table 27.4), and within the subgenus *Viannia* are the *L. braziliensis* complex (Table 27.5) and the *Leishmania guyanensis* complex (Table 27.6). It is very likely that taxonomy will continue to change as molecular methods reveal genetic differences and similarities among the various genera and species. Common names for New World CL include uta (Peru), dicera de Baurid (Brazil), chiclero ulcer or bay sore (Mexico), and pian bois or forest yaws (Guyana). Collectively, this group has also been called American CL. This disease is quite old and has even been depicted on pottery from Peru and Ecuador that has been dated ca. AD 400 to 900.

Figure 27.11 New World cutaneous leishmaniasis: multiple ulcers and scarring, Peru (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). doi:10.1128/9781555819002.ch27.f11



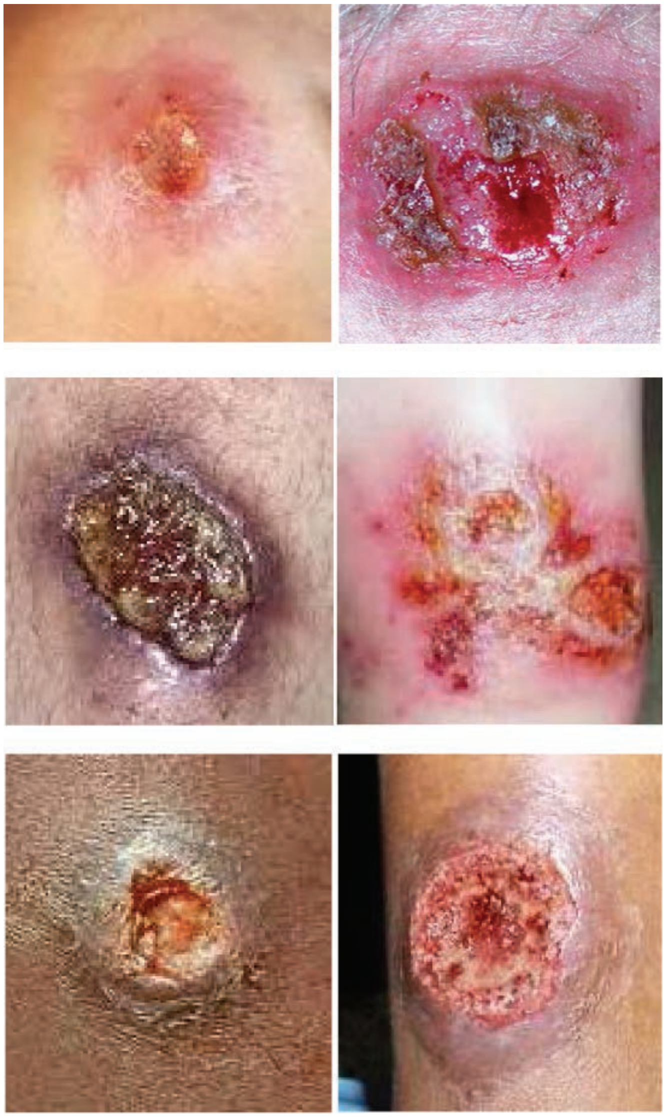


Figure 27.12 New World cutaneous leishmaniasis: six examples of cutaneous lesions. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch27.f12

TABLE 27.4 New World cutaneous leishmaniasis subgenus *Leishmania*, complex *Leishmania mexicana*

Species and sandfly vector	Human disease and lesion	Geographic distribution	Important hosts	Comments
L. mexicana: *Lutzomyia olmeca*, *L. anthophora* (Texas and Arizona)	Cutaneous leishmaniasis, chiclero ulcer	Belize, Guatemala, Honduras, Costa Rica, Mexico, Texas, Arizona (Panama, South America questionable); Southern Plains of Texas (wood rat [*Neotoma micropus*]); Arizona (white-throated wood rat [*Neotoma albigula*])	Forest rodents	Prolonged exposure of "chicleros," who live in forest collecting chewing gum latex from chicle trees (30% infected during first year); timber cutters, road builders, agricultural workers
L. venezuelensis: *Lutzomyia olmecabicolor*	Cutaneous leishmaniasis	Venezuela (humans only)	Humans	Poor long-term growth in blood agar medium; wild mammalian host not yet discovered; found in horses and the domestic cat
L. garnhami: *Lutzomyia youngi* (not confirmed)	Cutaneous leishmaniasis	Venezuelan Andes	Humans, marsupial (*Didelphis marsupialis*)	Identical to *L. amazonensis* by isoenzyme profiles, but clear differences between nontranscribed rDNA intergenic spacer sequences; still difference of opinion regarding validity of species designation
L. amazonensis: *Lutzomyia flaviscutellata*	Cutaneous leishmaniasis, diffuse cutaneous leishmaniasis	Amazon region of Brazil, Bolivia, Colombia, French Guyana, Paraguay	Forest rodents (primary); marsupials, foxes (secondary)	Human disease uncommon; vector nocturnal and not very anthropophilic, lives in swampy areas with few inhabitants; DCL similar to that seen in Ethiopia (Old World); specific anergy to leishmanin (Montenegro) skin test
L. pifanoi *Lutzomyia flaviscutellata*	Diffuse cutaneous leishmaniasis	Venezuela (states of Yaracuy, Lara, and Miranda)	Humans	Some confusion regarding actual taxonomy; wild mammalian host not yet discovered; DCL similar to that seen in Ethiopia (Old World); specific anergy to leishmanin (Montenegro) skin test

Life Cycle and Morphology

The life cycle and organism morphology are essentially the same as for Old World CL (Fig. 27.2). However, in some cases, destructive oral, nasal, or pharyngeal lesions may develop, causing mucocutaneous disease. In New World leishmaniasis, the sand fly vector is in the genus *Lutzomyia* or *Psychodopygus* rather than *Phlebotomus*. The genus *Leishmania* is divided into its two subgenera, *Leishmania* and *Viannia*, depending on the location of the development of the promastigote within the sand fly. Promastigotes from the subgenus *Viannia* develop in the midgut and hindgut of sand flies, while promastigotes from the subgenus *Leishmania* develop in the anterior portion of the sand fly alimentary tract (39).

Clinical Disease

Cutaneous Leishmaniasis. The lesions of New World CL are very similar to those seen with Old World cutaneous disease. Weeks to months after infection, an erythematous, often pruritic papule develops at the bite site. This papule may become scaly and enlarge, developing a central ulcer surrounded by a raised margin. Disease progression at this point will vary depending on the species involved. Lesions may be single or multiple and usually occur on exposed areas of the body. Many lesions may self-heal over a period of approximately 6 months (Fig. 27.11); however, lesions on the ear occur in 40% of the patients and are chronic, lasting many years. Ulcerations on the ears may be quite destructive but cause the patient few problems. *L. mexicana* lesions are usually single, self-limiting cutaneous papules, nodules, or ulcers that are painless and are found on the face and ears (Fig. 27.12 and 27.13). Other infections that are usually associated with single cutaneous lesions include those due to *Leishmania colombiensis*, *Leishmania amazonensis*, *Leishmania garnhami*, *Leishmania lainsoni*, *Leishmania peruviana*, and *Leishmania venezuelensis*. Multiple lesions caused by spread along lymphatics are common with *L. guyanensis* infections, and subcutaneous

TABLE 27.5 New World cutaneous leishmaniasis, subgenus *Viannia*, complex *L. braziliensis*

Species and sandfly vector	Human disease and lesion	Geographic distribution	Important hosts	Comments
L. braziliensis: *Lutzomyia wellcomei*, *L. intermedius*, *L. pessoai*	Cutaneous leishmaniasis, mucocutaneous leishmaniasis	Brazil, Guatemala, Belize, Honduras, Nicaragua, Peru, Ecuador, Bolivia, Costa Rica, Panama, Colombia, Venezuela, Paraguay, Argentina	Forest rodents, humans	Vector feeds primarily during daylight hours; common in farming communities in newly cleared forest areas and in road construction and mining workers; also known as nariz de anta (tapir nose) and espundia
L. peruviana: *Lutzomyia peruensis*, *L. verrucarum*	Cutaneous leishmaniasis	Peru, western slopes of the Andes, inter-Andean valleys; 900–3,000 m above sea level	Humans, dogs	Frequent in school children, commonly resulting in extensive facial scars; ulcers self-healing; known as uta; in some villages 90% of people are infected or scarred
L. colombiensis: *Lutzomyia hartmanni*, *L. gomezi*, *Psychodopygus panamensis*	Cutaneous leishmaniasis	Colombia, Panama, Venezuela, probably into Brazil, Peruvian lowlands	Humans, single isolate from the sloth (*Choloepus hoffmanni*)	Single to multiple ulcerating skin lesions; ML has not been seen
L. lainsoni: *Lutzomyia ubiquitalis*	Cutaneous leishmaniasis	Amazon region of northern Brazil, probably elsewhere	Humans, rodent (*Agouti paca*)	Multiple lesions have also been seen; ML has not been seen
L. shawi: *Lutzomyia whitmani*	Cutaneous leishmaniasis	Amazon region of northern Brazil, south of the Amazon River	Humans, monkeys, sloths, coatimundi	Multiple lesions have also been seen; ML has not been seen
L. naiffi: *Psychodopygus ayrozai*	Cutaneous leishmaniasis	Brazilian states of Pará and Amazonas	Humans, nine-banded armadillo (*Dasypus novemcinctus*)	Multiple lesions have also been seen; ML has not been seen

lymphatic nodules can be seen with *Leishmania panamensis*; the lesions can mimic those seen in sporotrichosis. The differential diagnosis of New World CL includes sporotrichosis, blastomycosis, yaws, syphilis, cutaneous tuberculosis, *Mycobacterium marinum* infection, and dermatologic cancers.

Mucocutaneous Leishmaniasis. ML is most common in the jungles of Amazonia (especially in southern Brazil), but cases have been reported throughout the Americas. Cutaneous lesions caused by *L. braziliensis* usually require a longer period for self-cure, taking up to 18 months or longer, and they can begin with lymphadenopathy. Approximately 1 to 3% of patients infected with *L. braziliensis* develop metastatic spread of the organisms to the nasal, pharyngeal, and buccal mucosa (ML). ML, also known as espundia, may occur months to years after the initial cutaneous lesion has healed partially or completely.

TABLE 27.6 New World cutaneous leishmaniasis, subgenus *Viannia*, complex *Leishmania guyanensis*

Species and sand fly vector	Human disease and lesion	Geographic distribution	Important hosts	Comments
L. guyanensis: *Lutzomyia umbratilis* (high infection rate, up to 7%)	Cutaneous leishmaniasis	Brazil north of Amazon River, Guyanas; range extends into Ecuador, Venezuela, lowland forests of Peru	Humans, two-toed sloth, lesser anteater, rodents, opossums	Reservoir infections inapparent, organisms in normal skin and viscera; vector lives in forest canopy and tree trunks; timing right for deforestation (early-morning biting); Brazil nut and fruit collectors, topographers, visiting botanists, zoologists, tourists affected; also known as pian bois or forest yaws
L. panamensis: *Lutzomyia trapidoi*, *L. ylephiletor*, *L. gomezi*, *Psychodopygus panamensis*	Cutaneous leishmaniasis	Panama and the Canal Zone, western and central Colombia, Ecuador, Venezuela, Costa Rica, Honduras, Nicaragua	Humans, sloths (two-toed and three-toed), occasionally other animals	Frequent in American military personnel; rare cases of ML

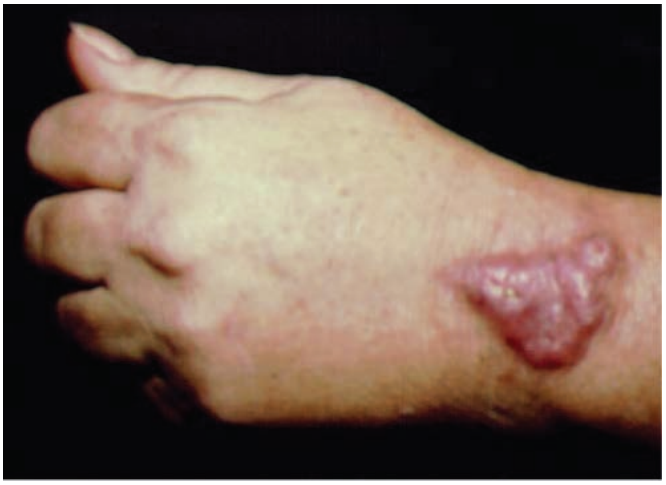


Figure 27.13 Large erythematous nodule with early ulceration on the left wrist, due to *Leishmania mexicana*. (Courtesy of Bonnie B. Furner.) doi:10.1128/9781555819002.ch27.f13

However, in many cases, mild mucosal lesions are present concomitantly with active cutaneous lesions. Mucosal disease tends to occur more frequently in males than females, and the patients are usually 10 to 30 years of age.

ML may begin with erythema and edema of the involved mucosal tissues, along with nasal inflammation, stuffiness, difficulty in breathing through the nose, and occasional bleeding. Following the early symptoms, ulcerations covered with a mucopurulent exudate may develop (Fig. 27.14). There is often severe erosion and destruction of the soft tissues and cartilage, leading to loss of the lips, soft parts of the nose, and soft palate. The lesions are chronic and progressive and can lead to death due to secondary infection or aspiration pneumonia. In the nonulcerative form, there may be hypertrophy of the tissues of the upper lip and nose, leading to the development of the "tapir nose." Unfortunately for diagnosis, parasites are few and difficult to find in tissues involved in ML. Although this disease can be extremely disfiguring, the mortality rate is low. New World ML mimics paracoccidioidomycosis, histoplasmosis, tuberculosis, syphilis, malignant tumors, and lethal midline granuloma.

Diffuse or Disseminated Cutaneous Leishmaniasis. DCL is an anergic form of CL in which neither humoral nor cell-mediated immune responses are functional but in which delayed hypersensitivity to tuberculin appears to be normal. DCL is seen most frequently in Venezuela, Brazil, Mexico, and the Dominican Republic. Individuals infected with *Lesihmania pifanoi* and 30% of those infected with *L. amazonensis* are likely to develop DCL (Fig. 27.15).

The parasites are found in the skin, and the disease is characterized by the formation of thickened skin in plaques, papules, or multiple nodules. Although lesions are usually seen on the face, nose, limbs, and buttocks, they can occur over the entire body (Fig. 27.16). The nodules are often described as soft and fleshy, while those of leprosy are generally more indurated. Although organisms have rarely been recovered from blood or bone marrow, there

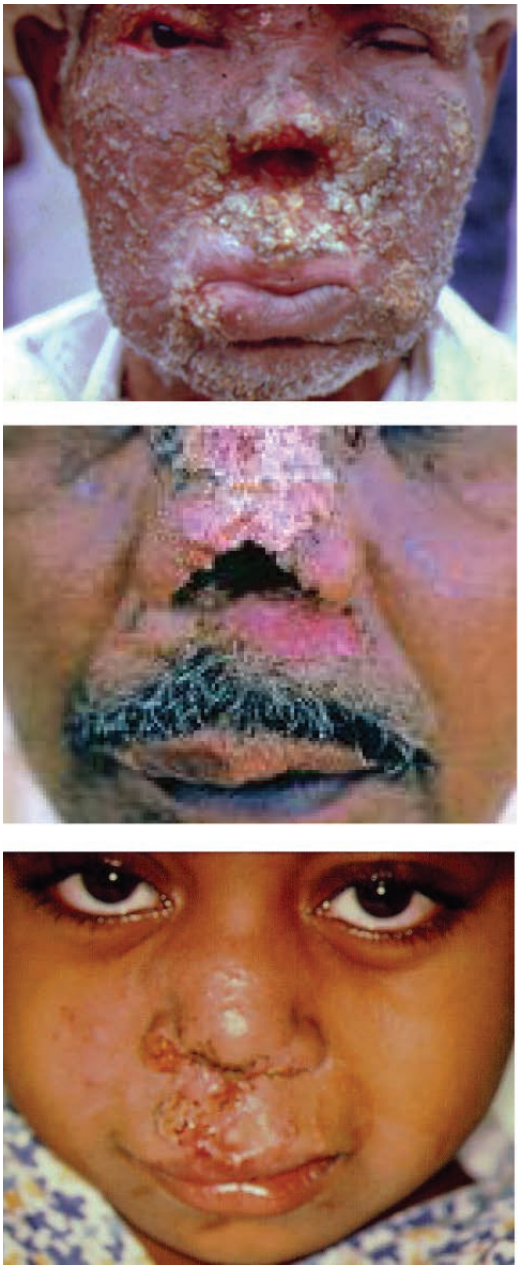


Figure 27.14 Mucocutaneous leishmaniasis, several examples of typical lesions (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). (Bottom image courtesy of Yoshihisa Hashiguchi.) doi:10.1128/9781555819002.ch27.f14

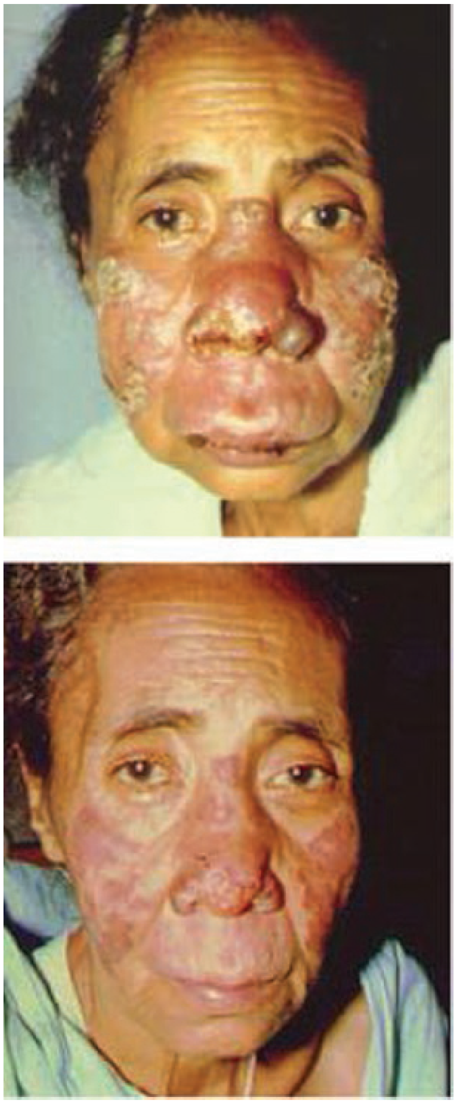


Figure 27.15 Mucocutaneous leishmaniasis. (Upper) Patient prior to therapy; (lower) patient after therapy (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). doi:10.1128/9781555819002.ch27.f15

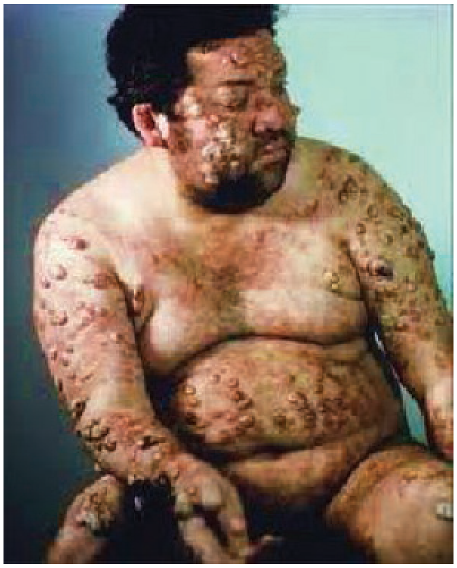


Figure 27.16 Diffuse anergic cutaneous leishmaniasis due to *Leishmania mexicana* in a patient of Dr. Convit (Venezuela). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch27.f16

are no visceral lesions associated with this form of leishmaniasis. Treatment is difficult, and the infection may last for the life of the patient. The LST remains negative unless treatment is effective and recovery occurs. Nodular DCL will have to be differentiated from lepromatous leprosy.

Diagnosis

In areas of the world where physicians are very familiar with leishmaniasis, the diagnosis may be made on clinical grounds. However, in locations where the disease is rare, the condition may not be recognized as leishmaniasis. Definitive diagnosis depends on demonstrating the amastigotes in tissue specimens or the promastigotes in culture (Table 27.7). More recent results suggest that PCR is a valuable tool for the diagnosis of leishmaniasis on a routine basis and can provide valuable epidemiologic information in areas of endemic infection. CL may have to be differentiated from a number of other lesions and diseases, including basal cell carcinoma, tuberculosis, various mycoses, keloid scarring, and lepromatous leprosy.

Specimen Collection

CL normally presents with no visceral involvement or systemic disease. The ability to detect parasites in aspirates, scrapings, or biopsy specimens depends on the number of amastigotes present, the level of the host immune response, the absence or presence of bacterial and/or fungal contamination within the lesion, and whether the specimen is collected from an active or healing lesion. If the patient has multiple lesions, specimens should be collected from the more recent or active lesions. These lesions should be thoroughly cleaned with 70% alcohol, and necrotic debris should be removed to prevent the risk of bacterial and/or fungal contamination of the specimen. Also, the specimen should be taken from the advancing margin of the lesion; the central portion of the ulcer contains nothing but necrotic debris unless portions of the lesion are taken below the crater area.

The specimen of choice would be a collection of several punch biopsy specimens taken from the most active lesion

TABLE 27.7 Features of New World human leishmanial infections related to demonstration of the parasite[a]

Organism	Disease type[b]	Humoral antibodies	Delayed hypersensitivity to leishmanin	Parasite present in clinical specimen	Self-cure	Recommended biopsy specimen
New World CL						
L. mexicana complex	CL	Present	Present	Present	Yes	Skin
	DCL	Variable	Absent	Abundant	No	Skin
L. braziliensis complex	CL	Present	Present	Present	Yes	Skin
	ML	Variable	Present	Few	No	Skin, mucous membranes
L. guyanensis complex	CL	Present	Present	Present	Slow	Skin
New World VL						
L. chagasi	VL	Abundant	Absent	Present	Possible	Bone marrow, spleen

[a] For culture, specimens must be collected aseptically, and in older lesions the parasites may be scant and difficult to recover. Animal isolation involves hamsters and culture media (Novy-MacNeal-Nicolle medium and Schneider's *Drosophila* medium with 30% fetal bovine serum). Serologic testing is most suitable for VL, has little value for CL, and has limited value for MCL. The Montenegro test (LST) is a delayed hypersensitivity reaction to intradermal injection of cultured parasites.

[b] Additional information on relevant diagnostic methods can be found in chapters 7 and 8.

areas. Biopsy specimens can be divided and used for cultures and touch preparations; some material should always be saved and submitted for routine histologic testing. The biopsy specimen can be used to make impression smears (touch preparations); these smears should be prepared after portions of the specimen have been placed in culture media by using a sterile technique. After any excess blood is cleaned off, a horizontal cut is made through the biopsy specimen core and the cut surface is gently touched to glass slides; multiple slides should be prepared. Once material has been set up for culture and touch preparations have been made, the remainder of the tissue can be sent to the pathology laboratory for routine processing.

Another option involving sterile techniques is to make a perpendicular slit in the margin of the lesion. After blood is removed using sterile gauze, the blade can be used to scrape the inner walls of the slit. Material can be inoculated into culture media and can be smeared onto several glass slides; multiple slides are recommended to increase the chances of seeing the parasites. Fine-needle aspiration can also be performed with a sterile syringe containing sterile saline (0.1 ml) and a 26-gauge needle. The needle is inserted into the outer border of the lesion and rotated several times, after which saline is injected and tissue fluid is aspirated back into the needle. Material can be submitted for culture, and/or smears can be prepared for staining. Additional techniques and specific methods can be found in chapters 6 to 8.

Specimen Staining. All smears to be Giemsa stained should be air dried and fixed with 100% methanol prior to staining. The amastigotes will be found within the macrophages or close to disrupted cells. In very early and older lesions, very few organisms may be present, so that prolonged examination of the slide may be required. Other blood stains can also be used with good morphologic results.

Culture Media. It is important to release the organisms from tissue prior to culture; mincing is recommended. However, tissue grinders are not recommended because of possible damage to the organisms. Culture media that have been used for the recovery and growth of the leishmaniae include Novy-MacNeal-Nicolle medium and Schneider's *Drosophila* medium supplemented with 30% fetal bovine serum (see chapter 8). Patient cultures should not be set unless the laboratory maintains specific organism strains for quality control checks. Both the control and patient cultures should be examined twice a week for the first 2 weeks and once a week thereafter for up to 4 weeks before they are reported as negative. Promastigote stages can be detected microscopically in wet mounts taken from centrifuged culture fluid. This material can also be stained with Giemsa stain to facilitate observation at a higher magnification.

Animal Inoculation. Animal inoculation can be helpful when only a small number of organisms are present; however, most laboratories no longer consider this approach practical. Golden hamsters are inoculated intranasally, and the major argument against this approach is that it may take several months before the animals give positive responses. A combination of tissue smears, culture, animal inoculation, and histopathology may be required for optimal diagnosis.

Skin Test. The LST (Montenegro test) is not species specific and must be interpreted with caution, particularly since some patients may have more than one type of leishmaniasis (Fig. 27.10). Positive reactions are generally seen in patients with cutaneous lesions; however, those with DCL give negative reactions.

Serologic Tests. Standard serologic tests are not particularly useful for the diagnosis of New World CL or ML. Low titers are seen, tests are likely to be negative in early disease, and overall interpretation is difficult at best. However, if a patient is positive, serial measurement of antibody titers may be helpful in tracking the outcome of therapy. The development and use of recombinant antigens for specific and sensitive serodiagnosis of CL have led to improved diagnostic results (40).

Treatment

In contrast to Old World CL, systemic chemotherapy is recommended for New World CL. Differences between the two types of CL tend to justify this recommendation and include cases with multiple lesions; the long-term, chronic nature of some infections; and the tendency for disease progression and mucosal involvement in the absence of chemotherapy. Time to healing has been improved in studies in which recombinant human granulocyte-macrophage colony-stimulating factor coupled with stibogluconate has been used (41). Studies also show that the combination of allopurinol and stibogluconate is superior to stibogluconate alone and can be used as an adjunct to stibogluconate or other oral agents in the treatment of CL (41). However, a combination of allopurinol and stibogluconate used for the treatment of ML provided no clinical benefit. It also appears that low-dose CL therapy using an antimony regimen (5 mg/kg/day for 30 days) may work just as well as the larger recommended dose (20 mg/kg/day for 3 to 4 weeks), particularly for children and the elderly (42).

The most promising drug found so far is an anticancer compound, miltefosine, which has undergone experimental and clinical trials and is 94 to 97% effective. However, the drug is contraindicated during pregnancy and may cause severe gastrointestinal side effects. Also, the cost is another limiting factor. Other drugs such as paromomycin, allopurinol, and sitamaquine have been reported to have variable cure rates. Because of these limitations, a combination therapy, preferably coupled with specific parasite enzyme inhibitors, may be the most effective approach (43).

In Mexico, CL occurs in 17 of 32 states and is considered a serious public health problem. Treatment with a localized current field radiofrequency device to generate precisely controlled heat as an alternative to expensive drug treatment appears to be effective and convenient for use in primary health care facilities. Lesions were first anesthetized with 1% lidocaine hydrochloride and moistened with normal saline solution. Treatment consisted of a single application of heat that produced 50°C for 30 s. After 4 weeks, 95% of 122 patients were totally cured (including those with infected ear cartilage), and at 8 weeks, 90% of 191 patients were cured (44).

ML is the most serious clinical form of CL and, once diagnosed, requires prolonged and close supervision of therapy; incomplete treatment can lead to severe sequelae and even death. For many years, mucosal involvement of leishmaniasis in America was attributed to *L. braziliensis* only. However, dissemination via blood or lymph or extension to mucosal tissue has been documented for *L. panamensis*, *L. guyanensis*, *L. mexicana*, and *L. amazonensis*. It is now accepted that most of the New World *Leishmania* species are capable of causing a wide spectrum of disease, depending on the immune status of the host, parasite strain, and anatomic exposure. The simultaneous presentation of both mucosal and active cutaneous lesions contrasts with the classical descriptions of ML caused by *L. braziliensis*, thus emphasizing the importance of early diagnosis of mucosal disease by examination of the mucosa in all patients with CL. In some cases, treatment with both conventional and experimental regimens may not be effective against ML (45).

Epidemiology and Prevention

***L. mexicana* Complex.** *L. mexicana* (Table 27.4) causes chiclero ulcer and is common throughout Mexico, Belize, Guatemala, and the southern United States. It is transmitted among forest rodents by vectors that are not particularly attracted to humans. However, prolonged exposure of many forest workers leads to a high infection rate in these groups. The range of endemic infection has now been expanded within the United States to include areas in Arizona that are farther north than the southern Texas site previously identified. Apparently, wood rats serve as the primary wild reservoir hosts in these two states.

L. venezuelensis is found in the Lara and Yaracuy States, Venezuela, and has been recovered from humans, horses, and domestic cats; no wild reservoir hosts have been identified. Although it develops rapidly in hamsters (animal inoculation), it does not grow well in the blood-based media routinely used for culture.

Recovery of *Leishmania garnhami* is limited to the Venezuelan Andes. Although the human is a known host, a single infection has also been recorded in the opossum, *Didelphis marsupialis*.

L. amazonensis has been found in Bolivia, Brazil, Colombia, French Guyana, and Paraguay and is likely to be found in other South American geographic locations where the appropriate vector is located. It is primarily an infection of forest rodents; however, marsupials and foxes can also be reservoir hosts. The vector tends to be nocturnal; this is probably the reason why human disease is not as common as with other species.

L. pifanoi is limited to the states of Yaracuy, Lara, and Miranda within Venezeula, and humans are the only known mammalian host. The wild mammalian host has not yet been identified.

***L. (Viannia) braziliensis* Complex.** Unfortunately, the geographic distribution of *L. braziliensis* (Table 27.5) is not well defined, but infections have been reported from most Latin American countries including Argentina, Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guyana, Guatemala, Honduras, Nicaragua, Panama, Paraguay, Peru, Suriname, and Venezeula. Many researchers think that it is unlikely that this broad a geographic range is accurate. The situation is even more confusing when ecologic and epidemiologic features of CL and ML differ in different regions. Humans are the main hosts, and wild hosts include sylvatic mammals such as rodents and marsupials. Infections have also been seen in dogs, mules, horses, and, rarely, cats. Extensive deforestation has been implicated in areas with suspected domestic animal transmission. Because there are questions regarding the reports of geographic range, it is difficult to accurately define vectors with proven transmission related to *L. braziliensis*. Human infections tend to be common in farming communities that develop along with deforestation, road construction, and mining.

L. peruviana is found in Peru on the western slopes of the Andes, including the inter-Andean valleys, generally at 900 to 3,000 m above sea level. It probably has a wider geographic range than reported. Transmission occurs in mountainous areas with little vegetation, and wild reservoir hosts probably include rodents and marsupials. Suspect vectors have been identified, but confirmation is often difficult. Human infection is usually contracted in and around the dwelling, and in some villages, the combined rate of present and past infection reaches 90%.

L. colombiensis has been found in Colombia, Panama, and Venezuela but probably has a wider geographic range, extending into Brazil and Peru, where appropriate wild reservoir hosts and vectors are found. Humans are the main host; however, a single isolate has been found from a sloth in Panama.

L. lainsoni occurs in the Amazon region of northern Brazil; however, the geographic range probably extends beyond these limits, as does the wild reservoir, *Agouti paca*, and the sand fly vectors. Evidence indicates that the vector, *Lutzomyia ubiquitalis*, does not like human blood, probably accounting for the low infection rate with *L. lainsoni*.

Leishmania shawi is also found in the Amazon region of northern Brazil, south of the Amazon river. Humans are recognized hosts, and wild reservoir hosts include monkeys, sloths, and coatimundi, as well as probably a few others.

Leishmania naiffi infections have been recorded from the Brazilian states of Pará and Amazonas. Like many of the other species where the wild reservoir hosts and appropriate vectors are found beyond these recorded boundaries, it is strongly suspected that the infection also has a broader range into other areas of Brazil and surrounding countries. The only recorded wild reservoir host is the nine-banded armadillo, in which there is a high infection rate in what appears to be normal skin and viscera. Like *L. lainsoni*, the vector of *L. naiffi* does not prefer human blood, hence the low human infection rate. However, other vectors may be implicated and do prefer human blood.

***L. (Viannia) guyanensis* Complex.** *L. guyanensis* (Table 27.6) is a very common cause of CL in Brazil, north of the Amazon river, and the Guyanas. Infections have also been recorded from Ecuador, Venezuela, and the lowland forests of Peru. Humans are known hosts, as are wild mammalian reservoir hosts such as the sloth and lesser anteater; other rodents and opossums have also been implicated. Like the wild reservoir hosts of *L. naiffi*, large numbers of parasites are located in what appears to be normal skin and viscera. The vector thrives in primary forest land, rather than in secondary forests or plantings of nonindigenous trees, and there may be a high human infection rate in such areas. Disease tends to be common in forest workers.

L. panamensis is found in Panama and the Canal Zone and has been reported as causing frequent infection in American military personnel stationed in these areas, as well as in agricultural workers. It has also been found in western and central Colombia, Ecuador, Venezuela, Costa Rica, Honduras, and Nicaragua. Humans are known hosts, and the epidemiology is similar to that seen with *L. guyanensis*. The major wild reservoir host is the two-toed sloth; fewer infections have been found in two genera of the three-toed sloths that live in the forest canopy, as do the vectors.

Vaccines. Current control of leishmaniasis is based on chemotherapeutic treatments which are expensive, toxic, and associated with high relapse and resistance rates. Vaccination remains the best hope for control of all forms of the disease. The development of a safe, effective, and affordable antileishmanial vaccine is a critical public health priority. Extensive evidence from studies in animal models indicates that solid protection can be achieved by immunization with defined subunit vaccines or live-attenuated strains of *Leishmania*. However, to date, no vaccine is available despite substantial efforts by many laboratories (46). Although not currently available, research and potential trials continue to be undertaken for vaccine development.

Visceral Leishmaniasis: General Comments

VL is widely distributed and endemic in 82 countries (Fig. 27.17). The estimated incidence of VL worldwide is a minimum of 100,000 per year, with close to 200 million people at risk of infection globally. Over 90% of these patients come from three regions: (Eastern/Old World) the Ganges River basin in India, Bangladesh, and Nepal, and the East African countries of Ethiopia, Sudan, and Kenya; and (Western/New World) northeastern Brazil. Since the 1990s, southern Sudan and the state of Bihar in India have been epidemic centers. These areas typically have high infection rates and a high mortality.

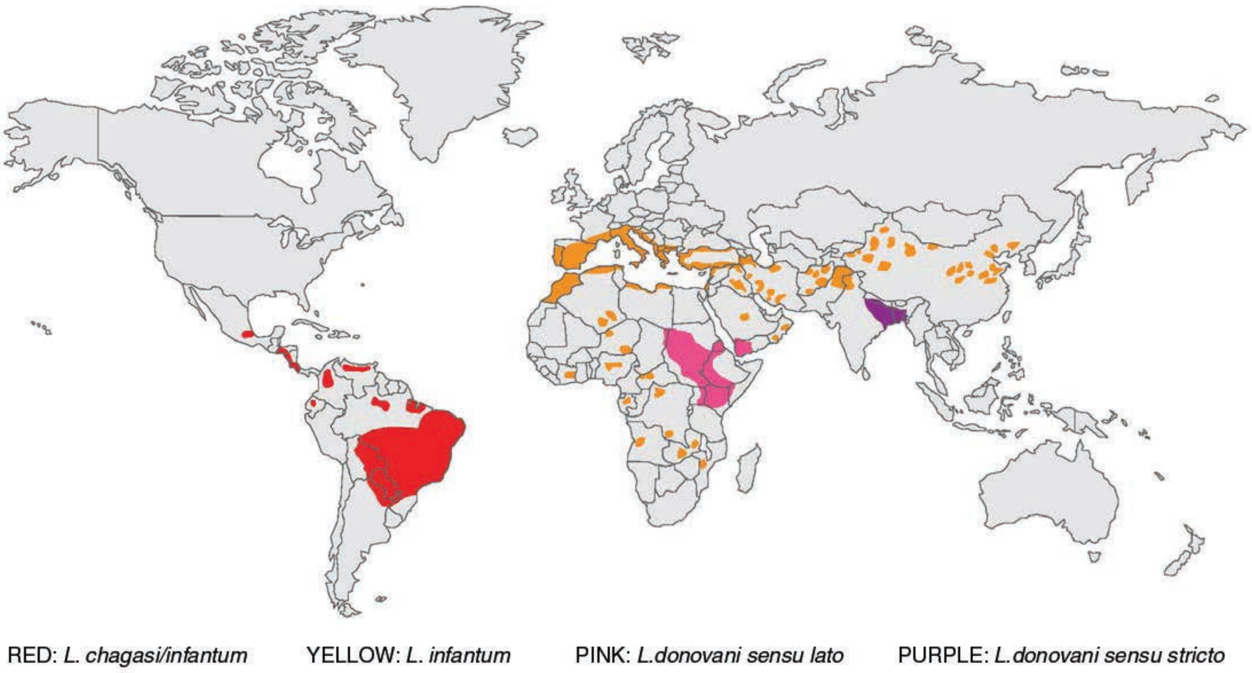
Old World (Eastern) Leishmaniasis: Visceral Leishmaniasis

VL (kala azar or dumdum fever) is caused by *L. donovani* and *L. infantum* in the Old World (Fig. 27.17). *L. tropica* also causes classical kala azar in India and causes viscerotropic leishmaniasis in the Middle East. The two main species tend to cause similar diseases, although *L. infantum* mainly affects young children and has a greater tendency to cause lymph node enlargement. The course of the disease and outcome depend on the general health status of the patient at the time of infection; apparently malnutrition exacerbates *L. infantum* infection (47). Dermotropic strains of *L. infantum* are known to cause visceral infection in immunocompromised patients with AIDS. VL is very prevalent among AIDS patients in certain geographic areas (e.g., southern Spain), with a high proportion of cases being subclinical. Like other opportunistic infections, subclinical VL can be found at any stage of HIV-1 infection, but symptomatic cases appear mainly when severe immunosuppression is present. HIV-*Leishmania* coinfection is being seen more and more frequently in the Mediterranean basin, especially in Spain, France, and Italy.

Life Cycle and Morphology

The life cycle and morphology of the organisms are essentially the same as for those causing Old World CL (Fig. 27.2). However, amastigotes can be found throughout the body and are not confined to the macrophages of the skin. Organisms inoculated into the bite site by the sand fly are engulfed by tissue macrophages, which then enter the bloodstream and are carried to RE centers such as the bone marrow, spleen, and liver (Fig. 27.18).

Figure 27.17 Geographic distribution of the agents of visceral leishmaniasis (courtesy of reference 2).
doi:10.1128/9781555819002.ch27.f17


RED: *L. chagasi/infantum*
YELLOW: *L. infantum*
PINK: *L.donovani sensu lato*
PURPLE: *L.donovani sensu stricto*

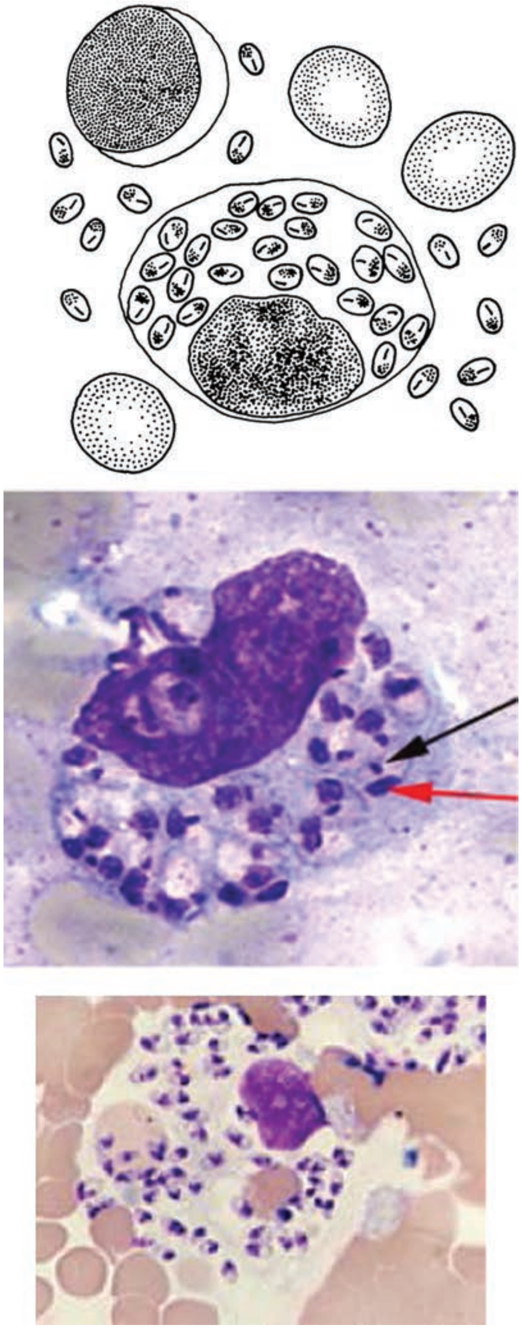


Figure 27.18 (Top) *L. donovani* amastigotes in bone marrow (illustration by Sharon Belkin). (Middle) Light-microscopic examination of a stained bone marrow specimen from a patient with visceral leishmaniasis, showing a macrophage containing multiple *Leishmania* amastigotes. Note that each amastigote has a nucleus (red arrow) and a rod-shaped kinetoplast (black arrow). Visualization of the kinetoplast is important for diagnostic purposes, to be confident the patient has leishmaniasis. (Bottom) *L. donovani* within bone marrow specimen.
doi:10.1128/9781555819002.ch27.f18

Clinical Disease

Visceral Leishmaniasis. Once the organisms have been carried to the bone marrow, spleen, and liver, a granulomatous cell-mediated immune response occurs that can result in subclinical disease and self-cure or in the clinical syndrome of VL (Table 27.8). The incubation period usually ranges from 2 to 6 months; however, it can take a few days or weeks to several years. When giving a history, many patients do not remember having a primary skin lesion. In areas of endemic infection, the onset may be gradual, with vague symptoms and a general feeling of ill health. The onset in naive patients (migrants, soldiers, or travelers to areas of endemic infection) may be acute, with high fever, chills, anorexia, malaise, weight loss, and, frequently, diarrhea. This syndrome can easily be confused with typhoid fever, malaria, or other febrile illnesses caused by bacteria or viruses. Death may occur after a few weeks or several years. Generally, untreated VL leads to death; secondary bacterial and viral infections are also common in these patients.

In areas where VL is endemic, many people develop a positive LST (Montenegro test) with no history of actual clinical disease; these individuals appear to be resistant to infection with *L. donovani*. The difference between an asymptomatic infection and subclinical disease is difficult to establish, particularly since many of these patients do not seek medical care. However, "viscerotropic" leishmaniasis may be more common than was previously thought and should be considered in individuals with unexplained mild symptoms (cough, malaise, chronic fatigue, abdominal pain, intermittent fevers, and diarrhea) in areas of endemic infection or in travelers with a history of exposure.

Immunology. With parasitization of RE cells, there is a hyperplasia of RE cells from the liver, spleen, bone marrow, lymph nodes, mucosa of the small intestine, and other lymphoid tissues. Hematopoiesis is initially normal and then becomes depressed, with a reduction in the life span of erythrocytes and leukocytes, causing anemia and granulocytopenia. RE cell proliferation may lead to massive hypertrophy of the liver and spleen, both of which may return to normal size after successful treatment (Fig. 27.19). In response to infection, there are decreases in T-cell blastogenesis production of interleukin-1 and -2, gamma interferon, and tumor necrosis factor. With successful therapy, these responses return to normal. Infection results in suppression of cell-mediated immunity, leading to uncontrolled reproduction of the parasite and dissemination. There is increased production of globulins, with high levels of IgG and IgM. The major target for the humoral immune response is heat shock proteins; Hsp70 was recognized in 92% of persons with VL (10). Circulating immune complexes involving IgA, IgG, and IgM can be found in the

TABLE 27.8 Old World visceral leishmaniasis

Species and sand fly vector	Human disease and lesion	Geographic distribution	Important hosts	Comments
L. donovani: *P. argentipes*, *P. orientalis*, *P. martini*	Visceral leishmaniasis, kala azar, post-kala azar dermal leishmaniasis	Indian subcontinent, East Africa	Humans	Characterized by irregular fever, splenomegaly, hepatomegaly, weight loss, pancytopenia, and hypergammaglobulinemia; statistically significant differences between number of organisms in peripheral blood taken during the evening and during the day (66 and 46%, respectively), which coincides with biting habit of sand fly vector (night biting) (45)
L. infantum: *P. ariasi*, *P. perniciosus*	Infantile visceral leishmaniasis, cutaneous leishmaniasis (rare)	Mediterranean basin, central and western Asia	Domestic dogs	Mainly affects young children and tends to cause lymph node enlargement; poor nutrition exacerbates infection

serum. Delayed hypersensitivity responses to skin test antigens are suppressed or absent. With resolution of infection, the delayed hypersensitivity reaction returns, and patients recovering from infection are considered immune to reinfection.

Clinical signs. Fever may occur at irregular intervals and, once established, may be seen as a double (dromedary) or triple fever peak each day. The patient is often weak and appears emaciated (Fig. 27.20). Common clinical signs include a nontender enlarged liver with Kupffer cells containing many amastigotes and little or no cellular reaction, a markedly enlarged spleen, femoral and inguinal lymphadenopathy, and occasional acute abdominal pain or vague abdominal discomfort. In progressive disease, splenic infarcts are common; in acute cases, the spleen is smooth and friable, while in more chronic cases, the spleen is firm. Hair changes (thinning, dryness, hypopigmentation, and loss of curl) may be seen. Edema of the legs, jaundice, petechiae, and purpura have also occasionally been seen.

Skin. Skin changes are often seen on the face, hands, feet, and abdomen, particularly in India, where patients acquire an earth-gray color; this darkening of the skin apparently gave rise to the name kala azar (black sickness). In Africa, skin lesions often accompany VL and may appear very different from one patient to another. Some lesions are diffuse, warty, and nonulcerated and contain variable numbers of amastigotes.

Mouth and nasopharynx. Patients with VL in the Sudan and sometimes in East Africa and India have mucosal lesions in the mouth and nasopharynx. The lesions may appear as nodules or ulcers; the gums, palate, tongue, or lips can be involved. Lesions in the nasal mucosa can result in perforation of the septum, while nasopharyngeal and laryngeal lesions are characterized by mucosal swelling and subsequent hoarseness. Parasites tend to be more numerous in oral than in nasopharyngeal lesions; however, they can be demonstrated in nasal and pharyngeal fluids.

Laboratory findings. Patients generally have anemia (normocytic and normochromic, assuming that there is no underlying iron deficiency), neutropenia, relative lymphocytosis, an almost complete absence of eosinophils, thrombocytopenia, and hypergammaglobulinemia (polyclonal B-cell activation). Liver enzyme levels may be mildly elevated, but elevations of bilirubin levels are uncommon. Malabsorption may be a severe problem in children with VL and can lead to complications or death.

Infections in immunocompromised individuals. During the last decade, leishmaniasis has emerged as one of the first and most important opportunistic infections in HIV-infected patients. Over 1,600 coinfection cases have been recorded, the majority from Europe, where 7 to 17% of HIV-positive individuals with fever have amastigotes. This finding strongly suggests that individuals infected with *Leishmania* spp. without symptoms will express symptoms of leishmaniasis if they become immunosuppressed. Based on the patient's general health status and prior exposure to the infection, leishmaniasis may be seen as an early opportunistic infection or as a complication late in the course of AIDS. In HIV-infected patients, VL occurs in late stages of HIV disease and often has a relapsing course (24). Secondary prophylaxis reduces the risk of relapse. VL in the HIV-infected population should be included in the Centers for Disease Control and Prevention clinical category C for the definition of AIDS in the same way that other geographically specific opportunistic infections are included. The majority of AIDS patients present with the classical picture of VL, but asymptomatic CL, mucocutaneous leishmaniasis, DCL, and PKDL can be seen (these are usually caused by *L. infantum*). Cutaneous lesions of VL are being found increasingly frequently in patients with

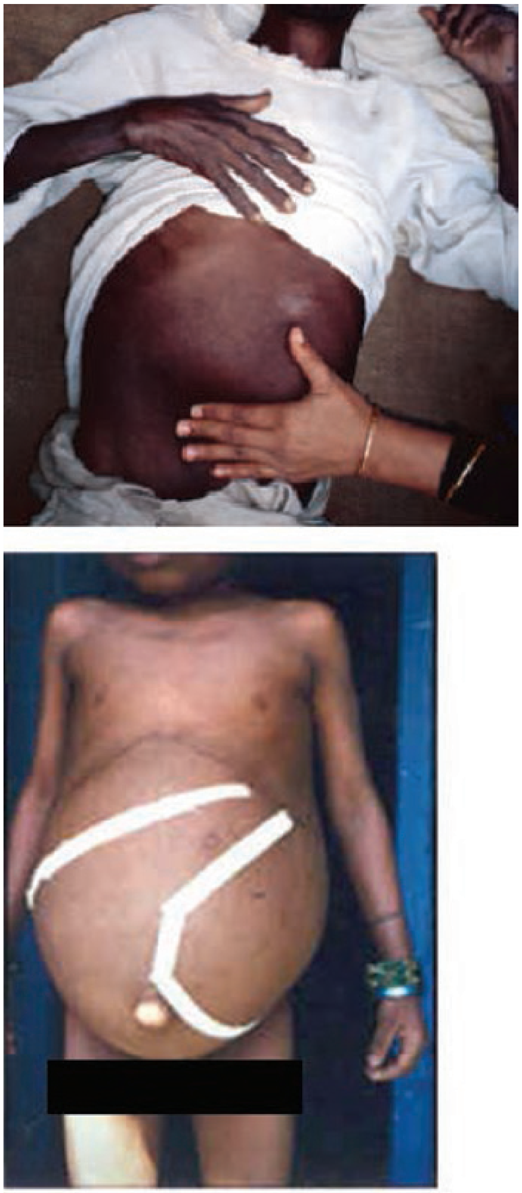


Figure 27.19 (Upper) Marked splenomegaly in a patient in lowland Nepal who has visceral leishmaniasis (VL) (courtesy of the CDC Public Health Image Library, C. Bern). (Lower) Marked splenomegaly and hepatomegaly in a patient with VL (courtesy of WHO, image from Philippe Desjeux).
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HIV infection, and their significance is still somewhat unclear. Lesions often do not present a uniform or specific appearance and have been seen as erythematous papules and hypopigmented macules on the dorsa of the hands, feet, and elbows; small subcutaneous nodules on the thighs; and erythematoviolaceous, scaly plaques on the face. Also, the digestive and respiratory tracts are often parasitized (21), as well as the pleura and peritoneum. Lesions can also be coinfected with other organisms, as evidenced by a case in which VL and *Mycobacterium avium-intracellulare* were seen in the same specimens from bone marrow and duodenal mucosa.

CD4 T-lymphocyte counts are usually <50/mm^3 and are almost always <200/mm^3. However, many physicians think that VL does not cause severe symptoms in these patients but is just one of several opportunistic infections that may coinfect AIDS patients. However, VL can manifest atypical aspects in HIV-positive patients depending on the degree of immunosuppression and should therefore be listed among AIDS-defining conditions. The most common symptoms are fever, splenomegaly, hepatomegaly, and pancytopenia.

Data from Spain provide some interesting outcome figures regarding coinfection compared with leishmanial infections in non-HIV-infected persons. In spite of a good initial response to treatment for VL, 60.6% of the HIV-coinfected patients had relapsed by the end of 1 year. Mortality from the first episode was 18.5%, and 24% died in the first month after diagnosis of any VL episode. The mean survival of the 29 patients who died was 10.27 months. Survival in patients with and without AIDS at the time of the first episode of VL was compared at 30 months and found to be 53.7 and 30.5%, respectively. A diagnosis of AIDS at the time of the first episode of VL and thrombocytopenia were the only risk factors found to be related to survival. Certainly in AIDS patients, VL is a recurrent disease that is highly prevalent and whose clinical course is modified by HIV (48). VL is very prevalent among HIV-1-infected patients in southern Spain, with a high proportion of cases being subclinical. Symptomatic cases appear mainly when severe immunosuppression is present. There is also an association between VL, the male sex, and intravenous drug use.

In HIV-1-infected patients with active VL, the sensitivity of a peripheral blood smear is about 50%, due to a high parasitemia in these patients. However, in patients with HIV-1 and subclinical VL, the sensitivity of a routine blood smear is <10% (49). Unfortunately, in these patients serology and *Leishmania* skin tests have low sensitivities and are not very helpful. Cultures have proven to be effective in diagnosing VL in patients who are coinfected with HIV-1 (50).

Post-Kala Azar Dermal Leishmaniasis. PKDL generally develops after apparent successful cure from visceral leishmaniasis and is confined to two geographically distinct zones, namely South Asia (India, Nepal, and Bangladesh) and East Africa, mainly Sudan. PKDL was first detected in patients with VL in India, occurs in up to 20% of these patients, has been called dermal leishmanoid, and is associated with *L. donovani* only. PKDL has also been

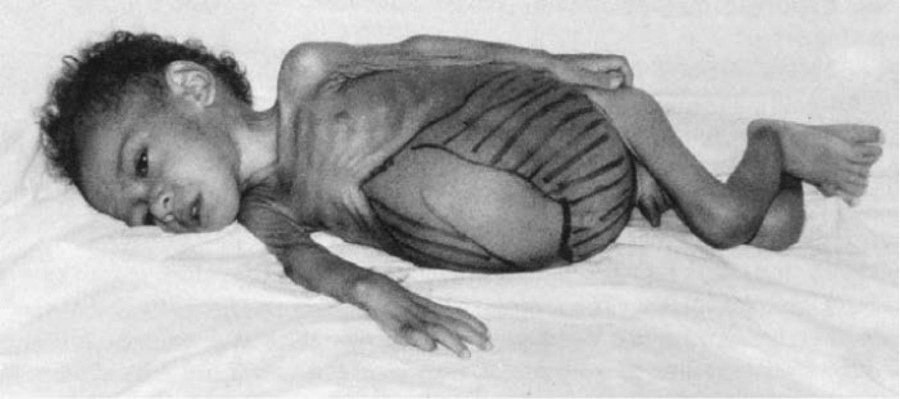


Figure 27.20 Cachexia (wasting) seen in severe visceral leishmaniasis (courtesy of Richard D. Pearson).
doi:10.1128/9781555819002.ch27.f20

seen in >50% of Sudanese VL patients treated in the current epidemic. In India, skin lesions may appear 2 to 10 years after successful therapy for VL; in East Africa, lesions appear within a few months; and in the Sudan, onset also occurs in about 2 months. Macules and papules usually appear first around the mouth and spread to the face and then to the extensor surfaces of the arms, the trunk, and sometimes the legs. In the beginning they look like small hypopigmented patches; these then enlarge and may progress to nodules that often resemble leprosy. The lesions are somewhat delicate but do not tend to ulcerate unless traumatized. In some cases, PKDL is seen in patients with no history of visceral disease (Fig. 27.21). Some patients with past or concomitant PKDL also have ocular lesions including conjunctivitis, blepharitis, and anterior uveitis; these conditions all respond to antimonial therapy, including steroid and atropine eyedrops.

The exact immune mechanisms of PKDL are still somewhat undefined, and the immunobiology of the Sudanese and South Asian PKDL differs. In Sudanese PKDL, because of the shorter time lag between cure from VL and development of PKDL, the disease-associated immune involvement mimics immune reactivation after cure from VL. Peripheral blood mononuclear cells from Sudanese PKDL patients react and proliferate following induction by *Leishmania* antigens and secrete more IFN-γ, whereas inteleukin-10 was produced primarily from CD4$^+$ T cells. In contrast, South Asian PKDL is more chronic due to the longer gap between cure from VL and disease onset. Here, CD8$^+$ T cells predominate in lesions and circulation. Also, in Sudanese PKDL, the lesions regress spontaneously, whereas in South Asia, prolonged treatment is essential (51).

Treatment of PKDL with antimonials usually brings improvement; however, the skin changes persist indefinitely. The course of therapy is generally longer than that required for VL and may require 120 days. In Africa, patients with PKDL may self-cure. It is important to remember that these patients may serve as reservoirs of infection during interendemic cycles, since some patients with PKDL tend to have large numbers of parasites in the skin. Persistent lesions currently require daily injections of sodium stibogluconate for 2 to 4 months, and even then the treatment may not be successful. Treatment with liposomal amphotericin B (AmBisome) has been found effective and is considered less nephrotoxic than nonliposomal amphotericin B because it specifically targets the macrophages in which the *Leishmania* parasites develop. Apparently, there are also intrinsic differences in the antibodies generated in the sera from patients with PKDL and VL (52). Diagnostic methods would be identical to those used for the isolation and identification of amastigotes found in CL.

Diagnosis

A clinical diagnosis of Old World VL is often made based on clinical findings and local epidemiologic factors. However, confirmation of the diagnosis requires demonstration of the amastigotes in tissues or clinical specimens or the promastigotes in culture (Algorithm 27.1). Detection of parasite genetic material or antigen detection also suffices for confirmation. Leishmaniasis should be suspected in individuals who have resided in or traveled to areas where the disease is endemic. The diagnosis would be supported by findings of remittent fevers, hypergammaglobulinemia with anemia, circulating immune complexes, rheumatoid factors, weight loss, leukemia, and splenomegaly. The differential diagnosis of late-stage VL is limited to hematologic and lymphatic malignancies. The diagnosis of early disease is more difficult, and the differential would include malaria, African trypanosomiasis, brucellosis, enteric fevers, bacterial endocarditis, generalized histoplasmosis, chronic myelocytic leukemia, Hodgkin's disease and other lymphomas, sarcoidosis, hepatic cirrhosis, and tuberculosis. Also, patients with multiple myeloma and Waldenström's macroglobulinemia have monoclonal hypergammaglobulinemia. Mixed cryoglobulinemia secondary to VL has also been documented.

Blood. Examination of buffy coat smears shows that there is a significant difference in recovery between samples col-

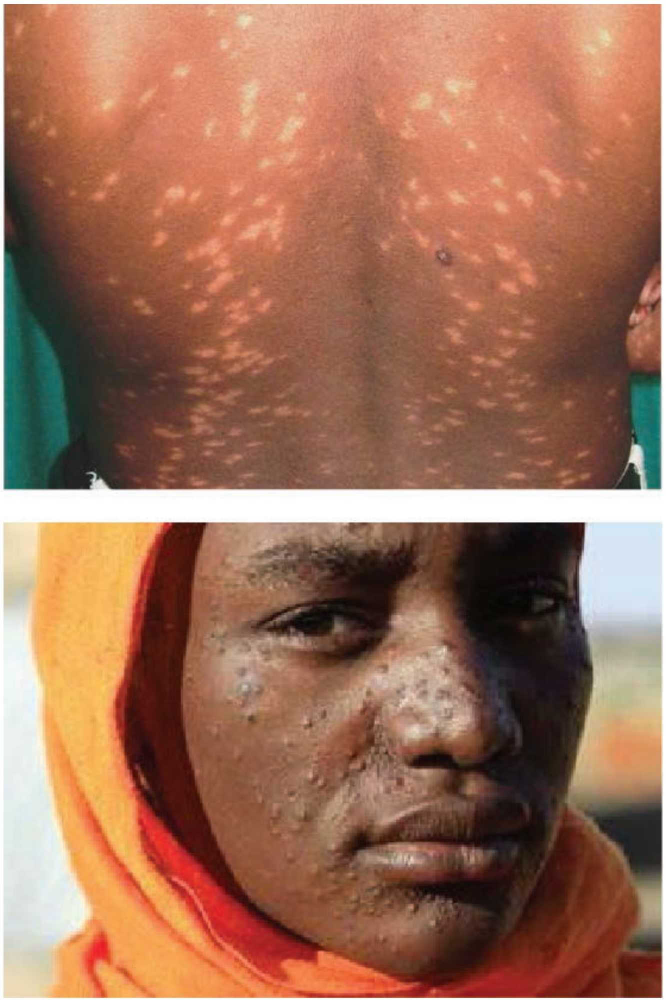
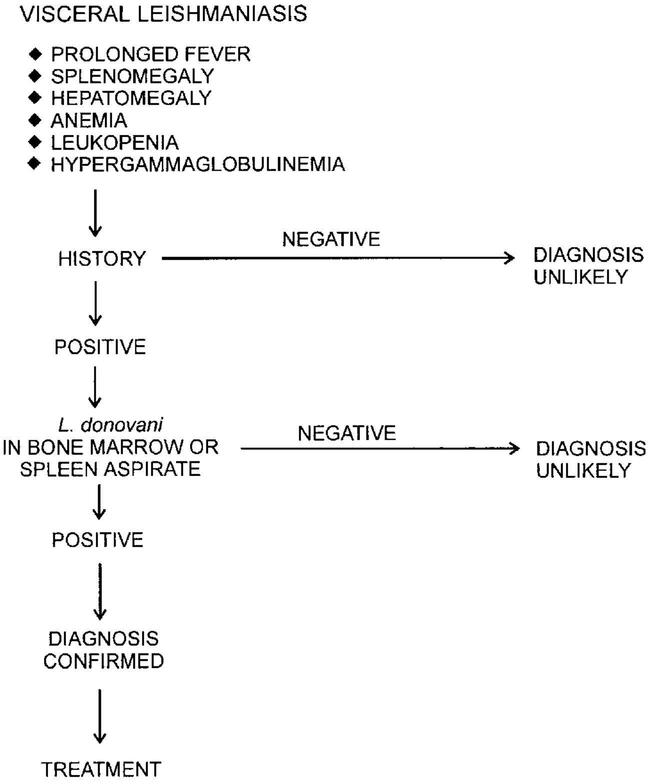


Figure 27.21 Post-kala azar dermal leishmaniasis (PKDL). (Upper) Notice the depigmented macules on the skin of a patient from India. This patient was treated for visceral leishmaniasis with miltefosine 7 years prior. He developed hypopigmented lesions about 3 years ago, which gradually became depigmented. With therapy the lesions disappeared. (Courtesy Koley S et al, *Indian J Dermatol* **58:**241, http://www.e-ijd.org/text.asp?2013/58/3/241/110848.) (Lower) Patient with rash all over her body, Eastern Sudan (courtesy of Reuters/Mohamed Nureldin Abdallah/Medecins Sans Frontières).
doi:10.1128/9781555819002.ch27.f21


VISCERAL LEISHMANIASIS
◆ PROLONGED FEVER
◆ SPLENOMEGALY
◆ HEPATOMEGALY
◆ ANEMIA
◆ LEUKOPENIA
◆ HYPERGAMMAGLOBULINEMIA
HISTORY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
L. donovani IN BONE MARROW OR SPLEEN ASPIRATE
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
DIAGNOSIS CONFIRMED
TREATMENT

Algorithm 27.1 Visceral leishmaniasis.
doi:9781555819002.ch27.Alg1

lected during the day (46%) and those collected during the night (66%) (53). Prior to an invasive procedure, blood specimens should be collected, keeping in mind the amastigote diurnal periodicity.

Tissue Aspirates. Although splenic puncture yields the highest rate of organism detection (98%), it carries a high degree of risk for the patient. Certainly deaths have occurred after this procedure, probably due to splenic laceration. Patients with coagulation disorders should not undergo splenic puncture. Other specimens include lymph node aspirates, liver biopsy specimens, sternal or iliac crest bone marrow, and buffy coat preparations of venous blood. Bone marrow aspirate is generally used in lieu of splenic puncture; however, smears from bone marrow usually contain fewer organisms, with a positivity rate of 80 to 85%. As discussed earlier in this chapter, it is important to prepare multiple smears for examination. Although amastigotes can be seen in buffy coat smears in Indian and Kenyan patients with VL, it is unusual to make the diagnosis by this method.

Culture and Animal Inoculation. Culture and animal inoculation studies are also helpful. Culture requirements, including specimen collection by an aseptic technique and the use of quality control strains, were discussed earlier in this chapter and are also addressed in chapter 8. A method for rapidly isolating promastigotes from the culture fluid has been developed using the stain acridine orange. This type of vital staining combines the advantages of direct microscopic examination with more accurate morphological imaging of the stained parasites. If available, hamsters should be inoculated intraperitoneally and killed after 2 to 3 months, and the liver and spleen should be checked for parasites (microscopic examination and/or cultures).

PCR and Other Antigen Detection Methods. PCR methods have excellent sensitivity and specificity for direct detection, for identification of causative species, and for assessment of treatment efficacy (34, 35, 54–59). A rapid immunochromatographic dipstick test using the recombinant RK39 antigen has become available for the qualitative detection of total anti-*Leishmania* immunoglobulins. The test uses antigen-impregnated nitrocellulose paper strips. Results from one study were very promising, with 100% sensitivity and 98% specificity (60). The RK39 strip test is ideal for rapid reliable field diagnosis of visceral leishmaniasis (61). The test has high sensitivity and specificity; however, it remains positive long after treatment (up to 3 years). For this reason, PCR is recommended as the more effective diagnostic approach (62).

Formol-Gel Test (Hypergammaglobulinemia). In patients with kala azar, there is a characteristic hypergammaglobulinemia, including both IgG and IgM. This fact has been used as the basis for a diagnostic procedure called the formol-gel test, which is used in areas of endemic infection. The test can be used as a screen; most patients with active disease are positive. A drop of formaldehyde is added to a test tube containing 1 ml of patient serum, which is observed for opacity and a stiff jelly consistency within 3 min to 24 h. Results also indicate that a specific IgE response is useful in the diagnosis of active VL and may be used to evaluate the response to treatment (63).

Skin Test. The Montenegro (LST) skin test is described above. In patients with active VL, this delayed hypersensitivity test is negative. However, within several months to a year after recovery, the skin test reverts to positive. It is of limited value because it becomes positive late in the course of disease, and false-positive results are common.

Serologic Tests. A number of serologic diagnostic methods have been developed worldwide, although they are not widely available. In general, sensitivities are >90% but specificities are lower. False-positive results can be seen in patients with other infectious diseases, in patients treated for VL (in whom antibodies persist for months), and in some asymptomatic VL patients. There are currently four tests in use, the direct agglutination test (DAT), the indirect fluorescent-antibody test, counterimmunoelectrophoresis, and enzyme-linked immunosorbent assay (ELISA). DAT appears to be the best choice as a diagnostic tool since it is very specific and does not require expensive equipment or reagents (64). However, patients may still have positive sera 1.5 to 5 years after treatment (65). DAT has also been adapted to filter paper sampling, and the filter paper eluates compared well with their homologous sera and correlated strongly with respect to antibody titers (66). DAT based on axenic amastigote antigen provided 100% sensitivity and specificity, making it particularly useful for diagnosis of macular PKDL cases, which are often missed by the RK39 strip test. Thus, DAT provides a simple, reliable, and inexpensive test for PKDL diagnosis with potential applicability under field conditions (67). However, newer studies using ELISA formats indicate excellent sensitivity and specificity and may be very helpful in diagnosis of early infections (68).

KEY POINTS—LABORATORY DIAGNOSIS

Old World (Eastern) Leishmaniasis: Visceral Leishmaniasis

1. For a complete laboratory diagnosis, microscopic examination of Giemsa-stained touch preparations and cultures is recommended, as well as PCR and animal inoculation if available. Other blood stains are also acceptable.
2. Due to potential problems with splenic puncture, bone marrow specimens may be recommended. Although not that productive, examination of buffy coat preparations is recommended prior to a more invasive procedure. When collecting blood specimens, remember the diurnal periodicity.
3. Multiple slides should be prepared for examination from aspirates and/or biopsy specimens.
4. Tissue imprints or smears should be stained with Giemsa stain (or one of the stains commonly used for blood smears) (see chapter 7).
5. Amastigote stages should be found within RE cells or close to disrupted cells.
6. If cultures are to be performed, specimens must be collected aseptically and control organism cultures should be set up at the same time (see chapter 8).
7. Cultures should be checked weekly for 4 weeks before they are declared negative.
8. Patients with kala azar have hypergammaglobulinemia; the formol-gel procedure may be helpful.
9. Serologic tests are helpful, and newer procedures demonstrate increased sensitivity and specificity; this should be considered if parasites cannot be detected in clinical specimens.

Treatment

Patients should receive supportive care, especially if they are malnourished or have other infections such as pneumonia, tuberculosis, or other chronic conditions. Since the mid-1930s, pentavalent antimony compounds have been the drugs of choice for the treatment of VL. However, with the first reports of primary treatment failures in the mid-1990s, additional drugs have been used and include lipid-associated amphotericin B for Mediterranean and Indian VL (52). VL in renal transplant recipients has been successfully treated with liposomal amphotericin B (AmBisome). The disadvantages of using Pentostam and Glucantime include the need for intravenous administration and hospitalization, cardiotoxicity, pancreatitis, treatment failures, and increasing resistance (69). The dosing regimen for treating VL is also associated with a high rate of serious side effects in HIV-infected patients (70). Therapy combining either paromomycin or allupurinol with pentavalent antimonials has also been used with some success.

There is no effective orally administered medication for any *Leishmania* infection. However, studies with miltefosine appear very promising. This drug is a phosphocholine analogue that affects cell-signaling pathways and membrane synthesis. When 100 mg of miltefosine was given per day (approximately 2.5 mg/kg of body weight/day) for 4 weeks, 29 (97%) of 30 patients with at least moderate *Leishmania* in their splenic aspirates were cured (71). Gastrointestinal side effects were frequent but were only mild to moderate in severity, and no patient discontinued therapy. Oral miltefosine and safe AmBisome along with better use of amphotericin B have been rapidly implemented in the last decade. A combination therapy will substantially reduce the required dose and duration of drug administration and reduce the chance of the development of resistance (41, 72).

Epidemiology and Prevention

The epidemiology of VL depends on a number of factors including the interaction between sand flies, reservoir hosts, and susceptible humans. In the two main areas of endemicity, the Ganges river basin and southern Sudan, high infection rates and many deaths have been documented during the last 20 years. Factors which affect the number of infections and the transmission include population movements often related to forced migration, poor general health, and poverty, all of which are seen in both geographic areas. The disease is a zoonosis, with the exception of India, where humans serve as the major reservoir host and the relevant vector is *P. argentipes*. Dogs serve as the main reservoir host around the Mediterranean, and the infection is transmitted through bites of infected sand flies such as *Phlebotomus perniciosus*, *Phlebotomus major*, *Phlebotomus simici*, and *Phlebotomus longicuspis*. Disease in this area is usually found in infants and children; young dogs are very susceptible to *L. infantum* and often die of the disease. Dogs are also important reservoirs in certain parts of China. In southern France and central Italy, infected foxes appear to be the reservoir, with *Phlebotomus ariasi* and *Phlebotomus perfiliewi* serving as vectors. Jackals also probably serve as reservoir hosts in the Middle East and central Asia. The epidemiology of VL in Africa is somewhat confusing, since the presence of epidemics suggests a human reservoir but dogs have also been found to be infected. The main vector in Kenya appears to be *Phlebotomus martini*, and in the Sudan the vector is *Phlebotomus orientalis*.

L. donovani occurs in Africa and Asia, and in areas where transmission is somewhat sporadic, wild canids and various rodents are natural reservoirs of the disease. *L. infantum* is seen in Africa, Europe, the Mediterranean area, and southwest Asia. Humans are accidental hosts, with dogs and other members of the *Canidae* acting as natural reservoirs (73).

Although the usual route of transmission is through the bite of infected sand flies, parasites can be transmitted in other ways including blood transfusion, sexual contact, congenital transmission, and occupational exposure. However, for the most part, transmission is through the normal vector cycle. Apparently, *L. infantum* can circulate intermittently and at low density in the blood of healthy seropositive individuals, who appear to be asymptomatic carriers; this certainly has implications for the safety of the blood supply in certain areas of the world, including southern France.

In areas where humans serve as the main reservoir host, treatment of active cases is likely to interrupt the cycle of transmission. Reservoir control has proven to be somewhat sporadic, and there is little indication that it has any impact on overall infection rates. Residual insecticide spraying, primarily for malaria vector control, was relatively successful; however, when spraying of DDT was discontinued, the incidence of VL began to increase again. Vaccines hold a great deal of promise, but currently no commercial products are available (74).

New World (Western) Leishmaniasis: Visceral Leishmaniasis

New World VL (sometimes called American VL [AVL]) is caused by *Leishmania chagasi* and has many similarities to Old World VL caused by *L. infantum*, including the fact that the infection is seen primarily in children and that both infections cause visceral and cutaneous disease.

Life Cycle and Morphology

The life cycle and morphology of *L. chagasi* are essentially the same as those of the species causing Old World VL (Fig. 27.2). Amastigotes can be found throughout the body and are not confined to the macrophages of the skin. Organisms inoculated into the bite site by the sand fly are engulfed by tissue macrophages, which then enter the bloodstream and are carried to RE cell centers such as the bone marrow, spleen, and liver.

Clinical Disease

L. chagasi causes subclinical infections and AVL, which is potentially fatal if not treated, and it has recently been

TABLE 27.9 New World (American) visceral leishmaniasis

Species and sand fly vector	Human disease and lesion	Geographic distribution	Important hosts	Comments
L. chagasi: Lutzomyia longipalpis	Visceral leishmaniasis, kala azar, post-kala azar dermal leishmaniasis, atypical cutaneous leishmaniasis	Most of Latin America (Argentina, Bolivia, Brazil, Colombia, Ecuador, Paraguay, Suriname, Venezuela, Guatemala, Guadeloupe, Honduras, Martinique, Mexico, El Salvador, Costa Rica)	Humans, dogs, foxes	Closely resemble *L. infantum* infections in Old World; fatal outcome unless treated; the term American visceral leishmaniasis (AVL) is preferred to kala azar (reserved for Indian VL)[a]

[a] Although there is some controversy about whether *L. chagasi* is indigenous to the Americas or whether AVL is due to *L. infantum* which was introduced into the New World by immigrants in post-Columbian times via infected dogs, there appears to be strong evidence for the former hypothesis.

associated with atypical cutaneous leishmaniasis (ACL) in Central America, particularly in Honduras (Table 27.9). Typically, AVL is associated primarily with malnourished children younger than 5 years, whereas the ACL presentations are seen mainly in children older than 5 years and young adults. Data indicate that this atypical disease presentation may be more common than was previously thought and may affect up to 10% of a local population (75). The fact that the same organism can cause AVL and ACL suggests that the host immune response may play a role in the outcome. The disease presentation includes nonulcerated cutaneous nodules.

Diagnosis

Confirmatory diagnosis depends on the demonstration of parasites, either amastigotes in stained smears of spleen, bone marrow, or lymph node aspirates or promastigotes in culture (Algorithm 27.1). Although tissue aspirates should contain many parasites, this is not always the case. If only a few are present, culture may not be successful. Intraperitoneal inoculation of aspirate material into hamsters is by far the most reliable means of isolating *L. chagasi*; however, there may be long delays before parasites are detected in the animals. PCR will probably become the test of choice, as with other types of leishmaniasis, but the practicality and cost issues mean that routine methods will continue to be the most likely approach, particularly since the majority of cases are seen in more remote rural areas.

Treatment

Although the treatment regimens are similar to those used for Old World VL, some patients are unresponsive to the recommended antimonial dosages; this has been seen in Bahia, Brazil. Alternatives would include increased dosages and/or the use of second-line drugs such as pentamidine. Unfortunately, serious side effects also increase when increased dosages are used. A review of available therapy for both Old World and New World VL is given in Table 27.10 and chapter 36.

Epidemiology and Prevention

The public health implications of widespread *L. chagasi* infections in Central and South America may be quite broad. Parasites that cause American CL (ACL) appear to be genetically very similar to those that cause AVL. This suggests that with diminished immune capacity, cutaneous infection may cause visceral disease, particularly since it is well known that this occurs in AIDS patients. ACL cases may also serve as reservoirs for vector transmission, leading to additional cases of visceral disease. Certainly treatment of these ACL patients would be recommended as a preventive measure for reduction of the number of potential future cases. Subcutaneous nodules are often misdiagnosed and treated inappropriately. Also, ACL may serve as a marker for the possible occurrence of AVL in a particular area and could be used as an indicator to more closely monitor for the presence of AVL.

In areas where humans serve as the main reservoir host, treatment of active cases is likely to interrupt the cycle of transmission. Reservoir control has proven to be somewhat sporadic, and there is little indication that it has any impact on overall infection rates. Residual insecticide spraying, primarily for malaria vector control, was relatively successful; however, when spraying of DDT was discontinued, the incidence of AVL began to increase again. Vaccines hold a great deal of promise, but currently no commercial products are available. As the incidence of disease continues to increase, particularly in Brazil, the importance of reviewing procedures for acceptance of blood donors and subsequent transfusions is becoming more relevant and important. Currently, blood is not routinely monitored for antibodies; however, serologic studies would serve as a first approach to the study of transmission of leishmanial organisms through blood transfusion. A seroreactivity of 9% has been found, increasing to 25% in a periurban focus of AVL infection (76). In another study from the midwest region of Brazil, 15.6% of samples tested were positive for antibodies to *Leishmania* sp. (77).

TABLE 27.10 Specific drugs for the treatment of visceral leishmaniasis (see also chapter 36)[a]

Drug	Dosage	Side effects	Comments[a]
Amphotericin B deoxycholate Lipid-associated amphotericin B Liposomal amphotericin B (AmBisome) Cholesterol dispersion (Amphotec) Amphoterican B lipid complex (Abelcet) **Liposomal amphotericin B is the drug of choice. The total dose administered seems to be more important than the number of infusions or duration of therapy.**	Slow i.v. over 4–6 h, starting at 0.1 mg/kg daily, increasing to 1 mg/kg every 2 days until total dose of 20 mg/kg has been given; AmBisome regimen is 3 mg/kg daily on days 1–5, sixth dose on day 14, seventh dose on day 21 (total dose is 21 mg/kg); regimen for immunosuppressed patients is 4 mg/kg daily on days 1–5, 10, 17, 24, 31, and 38 (total dose of 40 mg/kg); in this group relapse is common	Fever, chills, thrombophlebitis, renal insufficiency, anemia, hypokalemia, anorexia, nausea; caution when used on patients previously receiving SbV, who may be predisposed to cardiac events; for treatment of patients with VL within the United States, AmBisome is recommended and should be used unless contraindicated	Used in cases of VL resistant to SbV compounds; cure rates in VL clinically resistant to SbV and pentamidine approach 100%; all drugs available in the U.S. have been approved for other conditions; AmBisome now also approved for VL; AmBisome effective and safe for VL (Brazil, India, Kenya, Mediterranean); lipid-associated amphotericin B preparations probably not more effective than amphotericin B deoxycholate but can be given at higher doses with less toxicity; also more expensive
Pentavalent antimonials (SbV) Sodium stibogluconate (SSG) (Pentostam) Sodium antimony gluconate Meglumine antimoniate (Glucantime)	Pentostam for VL (India, Kenya): 20 mg/kg/day (28 days Kenya,40 days India); upper limit of safe, daily dose not as relevant, with the exception of AIDS patients	i.m. injections painful; recommend slow injection with 23- to 26-gauge needle, dilution of daily dose 1:10 with 5% DW. Nausea, anorexia, abdominal pain, malaise, headache, arthralgias, myalgias, lethargy (beginning 7–10 days into therapy); VL patients tolerate SbV better than do CL or ML patients; symptoms resolve after therapy; serum amylase and lipase levels rise (peak at 7–14 days); may need to interrupt therapy for 5-fold increase in amylase and 15-fold increase in lipase levels; ECG abnormalities occur in 50% of patients receiving SSG	Variation in total antimony (Sb) content; cannot assume that one drug will work like another; lot variations may contribute to safety and efficacy differences; storage conditions important (4°C in the dark optimal)
Miltefosine	2.5 mg/kg/day PO (max 150 mg/day × 28 days	The drug is contraindicated in breastfeeding and pregnant women; a negative pregnancy test before drug initiation and effective contraception during and for 4 months after treatment is recommended	Miltefosine (Impavido) is manufactured in 10- or 50-mg capsules by Paladin (Canada) and is available in the U.S. from the manufacturer through an investigative drug application from the FDA.
Pentamidine (as isethionate or methane sulfonate salt)	4 mg/kg daily 3 times/wk for 24 injections (8 wk); regimens of 4–6 mo have also been used	Pain, induration, sterile abscesses at injection site; hypotensive reactions if drug given too quickly; hypoglycemia diabetes may occur; high-dose, long-duration therapy more toxic than SbV and amphotericin B, giving it a limited role in treatment of VL	Provided as sterile powder, must be dissolved in DW; administered i.m. or slow i.v.; tends to be effective in Kenya with little toxicity; resistance in India now common; addition of SbV to pentamidine does not increase the cure rate
Aminosidine (paromomycin)	Parenteral (Kenya): 15 mg/kg/day for mean of 19 days (79% cure); parenteral (India): 20 mg/kg/day for 21 days (97% cure); accepted monotherapy for Indian VL at 16 mg/kg/day for 21 days	Possible auditory nerve damage and renal damage; vertigo and pancreatitis when given i.v.	Aminoglycoside with broad antiparasitic activity; if used in combination with standard doses of SbV, shorter duration (17–20 days) possible (Kenyan, Sudanese, Indian VL)
Immunotherapy	Has been used as monotherapy and adjunct therapy in SbV-resistant and primary VL in Kenya, Brazil, and India	Fever, chills, myalgias, fatigue, headache, depression	IFN-γ should be considered when faced with SbV-resistant VL and in selected cases of relapse; expensive (1, 7, 28, 30, 33)

[a] DW, distilled water (sterile); IFN-γ gamma interferon; i.m., intramuscular; i.v., intravenous; ECG, electrocardiogram; SbV, pentavalent antimonials; SSG, sodium stibogluconate.

Control programs for AVL often include elimination or treatment of infected dogs. Although attempting to remove seropositive dogs is insufficient as an eradication measure, data suggest that elimination of the majority of seropositive dogs may affect the cumulative incidence of seroconversion in dogs temporarily and may also diminish the incidence of human cases of AVL. The effectiveness of the use of vaccines plus repellents in preventing infection and subsequent disease development should be assessed by means of large-scale, randomized controlled field trials because this combined strategy may become the next frontier in the control of canine leishmaniasis (Fig. 27.22).

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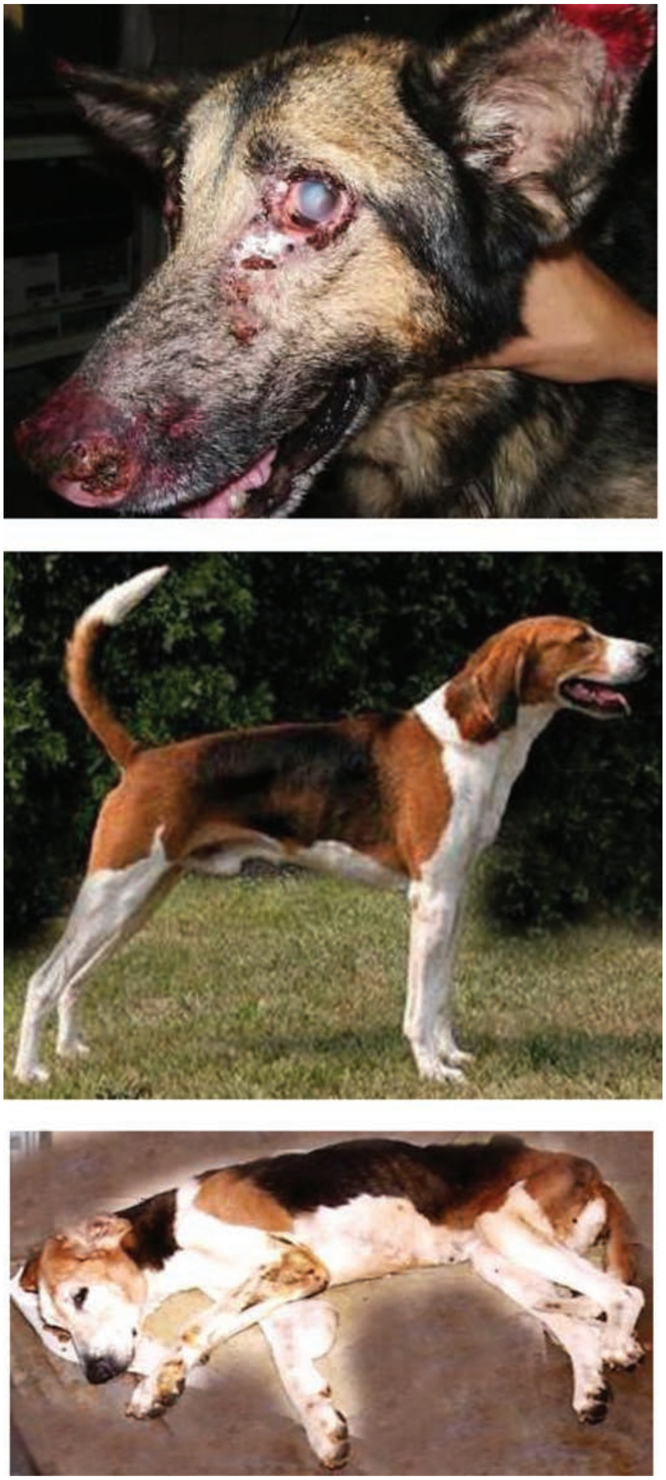

Figure 27.22 (Top) Adult German shepherd with clinical appearance suspect of cutaneous leishmaniasis (courtesy of Global Health Vet, image by Gaël Lamielle, http://globalhealthvet.com/2010/10/05/working-in-morocco-recurring-leishmaniasis-in-a-canine-patient/). (Middle) Adult American foxhound. (Bottom) Adult American foxhound. Previously considered an exotic disease, canine leishmaniasis caused by *Leishmania infantum* has been detected within the foxhound population in the United States and parts of Canada. *L. infantum* is the etiologic agent of visceral leishmaniasis in many areas of the world and dogs are considered a major reservoir host for human *Leishmania* infections. doi:10.1128/9781555819002.ch27.f22

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28

Trypanosomiasis

African trypanosomiasis

Trypanosoma brucei gambiense

Trypanosoma brucei rhodesiense

American trypanosomiasis

Trypanosoma cruzi

Trypanosoma rangeli

Trypanosomes are hemoflagellates, which are transmitted by tsetse flies (*Glossina* spp.) to a wide range of mammalian hosts (1) (Fig. 28.1). They cause serious medical problems for humans and livestock. Trypanosomes belong to the family *Trypanosomatidae*, which live in the blood and tissues of their human hosts (Table 28.1). The African trypanosomes belong to the subgenus *Trypanozoon* and as a group are referred to as the *Trypanosoma brucei* complex. *T. brucei brucei* strains are parasites of domestic and wild animals and are not known to be infectious for humans. Human infections are caused by *T. brucei gambiense* (West African trypanosomiasis) and *T. brucei rhodesiense* (East African trypanosomiasis). Infections caused by *T. brucei rhodesiense* are much more fulminant than those caused by *T. brucei gambiense*, the parasitemia is much higher, and the disease has a much faster progression. It is not possible to differentiate the three trypomastigotes of *T. brucei gambiense*, *T. brucei rhodesiense*, and *T. brucei brucei* on the basis of morphology, and all three organisms can be recovered in animal reservoir hosts. In the past, differentiation was based on clinical signs and geographic area of isolation. Differentiation is now based on isoenzyme characteristics and DNA and RNA methods in addition to the aforementioned factors. It is estimated that 60 million people in 36 countries are at risk, and there are approximately 50,000 new cases per year of African trypanosomiasis (2). Based on available information from western and central Africa, there appears to be no increased risk of African trypanosomiasis among those infected with HIV; however, this has not been totally confirmed. There are also unanswered questions concerning disease presentations and diagnostic test results in patients who are coinfected with both organisms. Human African trypanosomiasis is characterized by a polyclonal B-cell activation, constituting a risk for false-positive reactions to diagnostic tests, including HIV tests. Unless tests have been validated for interference with African trypanosomiasis, HIV diagnosis using standard algorithms in untreated trypanosomiasis patients should be avoided. Validated combinations of three HIV rapid tests can increase specificity (3).

Trypanosomes infecting humans in the Americas belong to the subgenus *Tejaraia* (*T. rangeli*) and *Schizotrypanum* (*T. cruzi*). *T. rangeli* infections are asymptomatic, with no evidence of pathology; however, *T. cruzi* infections (American trypanosomiasis) can cause considerable morbidity and mortality. Chagas' disease is caused by the protozoan parasite *T. cruzi*, which affects as

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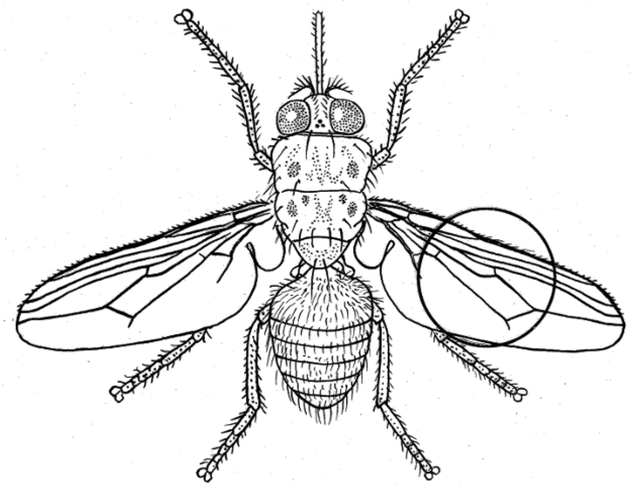


Figure 28.1 Tsetse fly (*Glossina* sp.), vector of African trypanosomiasis; the typical "hatchet" cell is seen within the circle. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch28.f1

many as 10 million to 16 million people in North and South America. However, due to the recent emigration of people from areas of endemicity, cases of Chagas' disease have been reported in the United States, Canada, Australia, Japan, and Europe, extending the range of this parasitic disease throughout the developed world.

African Trypanosomiasis

Trypanosoma brucei gambiense

Although the first descriptions of human sleeping sickness were made by European colonists in the late 19th and early 20th centuries, Atkins described a "sleepy distemper" in 1742 and David Livingstone described a "fly disease" in 1857 (2). In 1901, *T. brucei gambiense* was seen in the blood of a European steamboat captain working on the Gambia River. After Forde's discovery in 1901, Dutton in 1902 proposed the name *Trypanosoma gambiense*. Although a trypanosome was discovered in 1903 in the spinal fluid of a patient suffering from sleeping sickness and Castellani named it *Trypanosoma ugandense*, it was soon clear that the two organisms were identical. By 1909, the mode of transmission by the tsetse fly, *Glossina palpalis*, was confirmed. The primary area of endemic infection with *T. brucei gambiense* coincides with the tsetse fly belt through the heart of Africa, where 300,000 to 500,000 people may be infected in western and central Africa (Fig. 28.2). The disease remains largely unchecked due to the sophisticated biological defense mechanisms adopted by the parasite, its tolerance to therapy, and the socioeconomic realities in a region where priorities may not include adequate surveillance and control (2).

Life Cycle and Morphology

Life Cycle. Trypanosomal forms are ingested by the tsetse fly (*Glossina* spp.) when a blood meal is taken (Fig. 28.3 and Fig. 28.4). Once the short, stumpy trypomastigote reaches the midgut of the tsetse fly, it transforms into a long, slender procyclic stage. The organisms multiply in the lumen of the midgut and hindgut of the fly. After approximately 2 weeks, the organisms migrate back to salivary glands through the hypopharynx and salivary ducts, where the organisms attach to the epithelial cells of the salivary ducts and then transform to their epimastigote forms. In the epimastigote forms, the nucleus is posterior to the kinetoplast, in contrast to the trypomastigote, in which the nucleus is anterior to the kinetoplast (Fig. 28.5). There is continued multiplication within the salivary gland, and metacyclic (infective) forms develop from the epimastigotes in 2 to 5 days. With development of the metacyclic forms, the tsetse fly becomes infective. During the act of feeding, the fly introduces the metacyclic trypanosomal forms into the next victim in saliva injected into the puncture wound. The entire developmental cycle in the fly takes about 3 weeks, and once infected, the tsetse fly remains infected for life.

Morphology. The trypanosomal (trypomastigote) forms can be found in the blood, cerebrospinal fluid (CSF), lymph

TABLE 28.1 Classification of trypanosomes infecting humans

Family			*Trypanosomatidae*
Genus			*Trypanosoma*
Subgenus	*Schizotrypanum*	*Tejaraia*	*Trypanozoon*
Species	*T.* (*S.*) *cruzi*	*T.* (*T.*) *rangeli*	*T.* (*T.*) *brucei*
Subspecies			*T.* (*T.*) *brucei gambiense* *T.* (*T.*) *brucei rhodesiense*

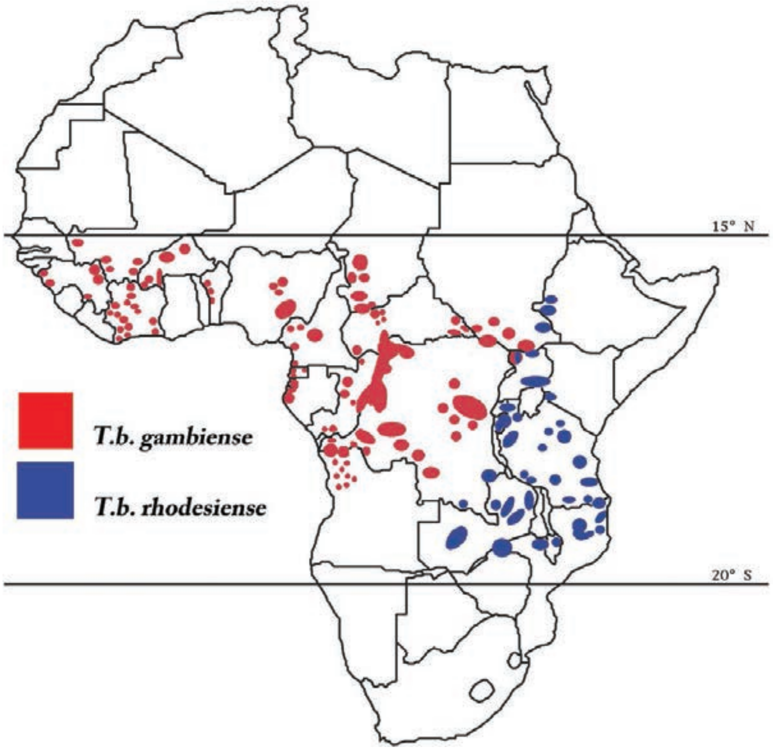
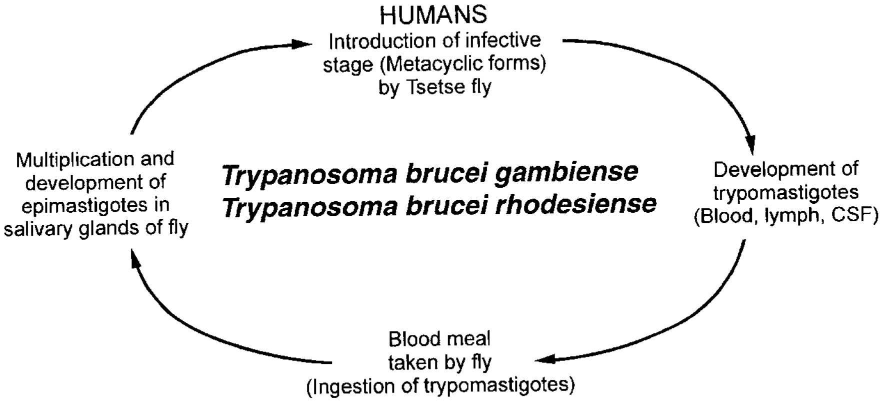

15° N
20° S
T.b. gambiense
T.b. rhodesiense

Figure 28.2 Distribution of trypanosomes in Africa. (Armed Forces Institute of Pathology map, modified from WHO Report Series 739, 1986; from reference 1.) doi:10.1128/9781555819002.ch28.f2

node aspirates, and fluid aspirated from the trypanosomal chancre (if one forms at the site of the tsetse fly bite). The trypomastigote forms multiply by longitudinal binary fission (Fig. 28.4). The organism is highly pleomorphic, having a variety of trypanosomal forms in the same blood smear. The forms range from long, slender-bodied organisms with a long flagellum (trypomastigote) that attain a length of 30 µm or more to short, fat, stumpy forms without a free flagellum that are approximately 15 µm long. The short, stumpy forms do not divide in the bloodstream but are the infective stage for the tsetse fly.

In blood, the trypanosomes move rapidly among the red blood cells (RBCs). An undulating membrane and flagellum may be seen with slower-moving organisms. The trypomastigote forms are 14 to 33 µm long and 1.5 to 3.5 µm wide (Fig. 28.6). With Giemsa or Wright's stain, the granular cytoplasm stains pale blue and contains dark blue granules and possibly vacuoles. The centrally located nucleus stains reddish. At the posterior end of the organism is the kinetoplast, which also stains reddish, and the remaining intracytoplasmic flagellum (axoneme), which may not be noticeable. The flagellum arises from the kinetoplast, as does the undulating membrane. The flagellum runs along the edge of the undulating membrane until the undulating membrane merges with the trypanosome body at the anterior end of the organism. At this point, the

Figure 28.3 Life cycle of *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*. doi:10.1128/9781555819002.ch28.f3


HUMANS
Introduction of infective stage (Metacyclic forms) by Tsetse fly
Development of trypomastigotes (Blood, lymph, CSF)
Blood meal taken by fly (Ingestion of trypomastigotes)
Multiplication and development of epimastigotes in salivary glands of fly
Trypanosoma brucei gambiense
Trypanosoma brucei rhodesiense

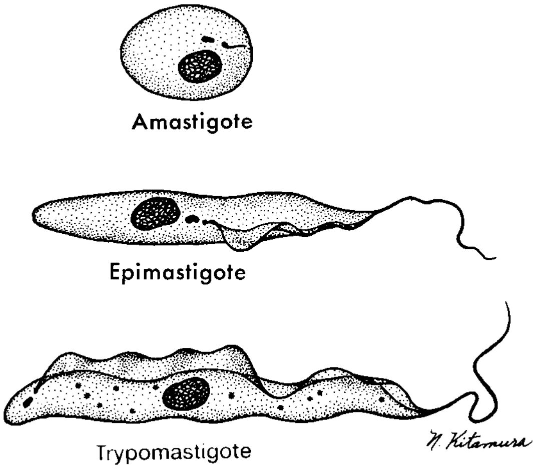


Figure 28.5 Life cycle stages of trypanosomes. Amastigotes have been confirmed in the life cycle of *Trypanosoma cruzi* as one of the stages in the human host, but this stage in humans in African trypanosomiasis is only suspected and not confirmed. (Illustration by Nobuko Kitamura.) doi:10.1128/9781555819002.ch28.f5

flagellum becomes free to extend beyond the body (Fig. 28.2). Although the trypomastigote is the diagnostic stage seen in clinical specimens, amastigotes have been seen in the choroid plexus in mice.

Antigenic Variation. A unique feature of African trypanosomes is their ability to change the surface coat of the outer membrane of the trypomastigote, helping to evade the host immune response. The trypomastigote surface is covered with a dense coat of approximately 10^7 molecules of the membrane-form variant surface glycoprotein (VSG). There are approximately 100 to 1,000 genes in the genome, responsible for encoding as many as 1,000 different VSGs. More than 100 serotypes have been detected in a single infection. It is postulated that the trypomastigote changes its coat about every 5 to 7 days (antigenic variation). This change is responsible for successive waves of parasitemia every 7 to 14 days and allows the parasite to evade the host humoral immune response (4). There is no evidence that the host immune system induces the VSG switches. During antigenic switching, VSG is both internalized by the trypomastigote and released or shed into the blood.

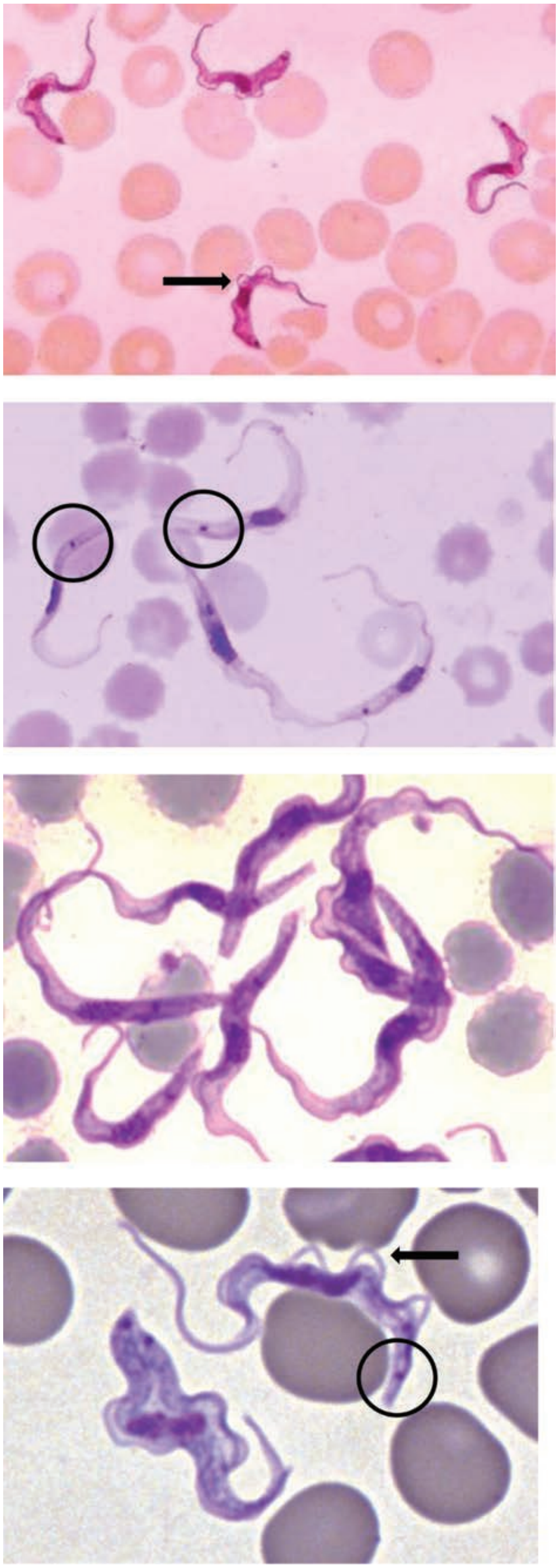


Figure 28.4 *Trypanosoma brucei gambiense* trypomastigotes in peripheral blood smears. Note the small kinetoplast (circle) and undulating membrane (arrows). Also, note in the second image, two of the trypomastigotes at the bottom appear to have just completed binary fission. Division can also be seen in the lower two images. (Bottom image courtesy of Armed Forces Institute of Pathology.) doi:10.1128/9781555819002.ch28.f4

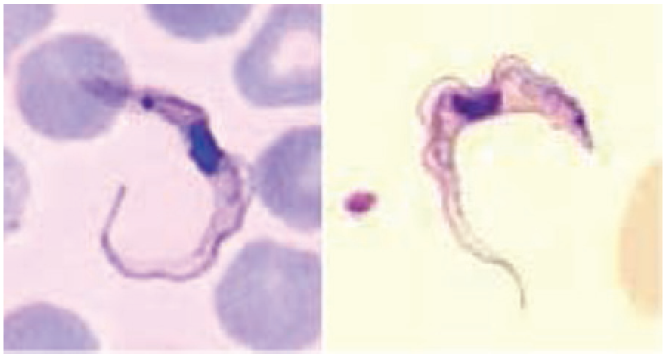


Figure 28.6 *Trypanosoma brucei gambiense* trypomastigotes in peripheral blood smears; note the undulating membrane.
doi:10.1128/9781555819002.ch28.f6

This shed VSG is most probably responsible for the immune dysfunctions noticed during infections. There is a release of gamma interferon (which stimulates parasite growth), suppression of interleukin-2, and hypergammaglobulinemia. Based on studies using *T. brucei*, bloodstream forms when aggregated in the presence of antibodies can subsequently disaggregate, and this mechanism may function to aid survival of the trypomastigotes in the presence of antibody in the host prior to the occurrence of a VSG switching event (5).

VSG is a 58-kDa glycophosphatidylinositol (GPI)-anchored glycoprotein, having predominantly an α-helical secondary structure, with highly variable sequences embedded at the N terminus and monoallelically expressed from a repertoire of hundreds of genes (4, 6). The disaggregation appears to be strictly energy dependent, and the organisms remain motile, metabolically active, and infective following this process.

Antigenic variation, however, is probably only one of several mechanisms enabling these parasites to thrive in spite of the host immune defenses. The ability to grow at high levels of gamma interferon, to avoid complement-mediated destruction, and to vary their susceptibility to a subclass of human high-density lipoproteins may also facilitate organism survival (7).

Genome. The genome of *T. brucei* has been defined with a complete analysis of the 11 megabase-sized chromosomes. The 26-Mb genome contains 9,068 predicted genes, including approximately 900 pseudogenes and approximately 1,700 *T. brucei*-specific genes. There are 806 VSG genes. Based on this research, a number of potential drug targets have been identified (8).

Clinical Disease

In general, African trypanosomiasis caused by *T. brucei gambiense* (West African sleeping sickness) has a long, mild, chronic course that ends fatally with central nervous system (CNS) involvement after several years' duration. This is unlike the disease caused by *T. brucei rhodesiense* (East African sleeping sickness), which has a short course and ends fatally within a year.

Stage I. After the host has been bitten by an infected tsetse fly, metacyclic trypomastigote stages are introduced into the skin, where they multiply and set up a local inflammatory reaction. A nodule or chancre at the site (3 to 4 cm) may develop after a few days (Fig. 28.7). However, this primary lesion will resolve spontaneously within 1 to 2 weeks. The chancre is seen frequently in white Europeans but rarely in patients indigenous to an area where the disease is endemic. Trypomastigotes may be detected in fluid aspirated from the ulcer. The trypomastigotes enter the bloodstream, causing a symptom-free low-grade parasitemia that may continue for months. This is considered stage I disease, where the patient can have systemic trypanosomiasis without CNS involvement. During this time, the parasites may be difficult to detect, even by thick blood film examinations. The infection may self-cure during this period without development of symptoms or lymph node invasion.

Symptoms may occur months to years after infection. The first distinct symptoms appear on invasion of the lymph nodes and are followed by the onset of remittent, irregular fevers with night sweats. Headaches, malaise, and anorexia frequently accompany the fevers. The febrile period of up to a week is followed by an afebrile period of variable duration and then another febrile period, and so on. Many trypomastigotes may be found in the circulating blood during fevers, but few are seen during afebrile periods. Lymphadenopathy is a consistent feature of Gambian trypanosomiasis. Enlarged lymph nodes are soft, painless, and nontender. Although any lymph node may be affected, posterior cervical regions are most frequently involved (Winterbottom's sign) (Fig. 28.7). Trypomastigotes can be aspirated from the enlarged lymph nodes. In addition to lymph node involvement, the spleen and liver become enlarged. With Gambian trypanosomiasis, the blood-lymphatic stage may last for years before the sleeping sickness syndrome occurs.

Transient edema is frequently seen and occurs in the face, hands, feet, and other periarticular areas. Pruritus is common, and in light-skinned individuals, an irregular erythematous rash, suggestive of erythema multiforme, can be seen. The rash occurs 6 to 8 weeks after the onset of infection and is typically located on the trunk, shoulders, buttocks, and thighs. It usually lasts only a few hours, and it appears and disappears during the febrile period. Patients may experience delayed sensation to pain (Kerandel's sign). Laboratory findings include anemia, granulocytopenia, reduction in platelet counts, increased sedimentation rate,

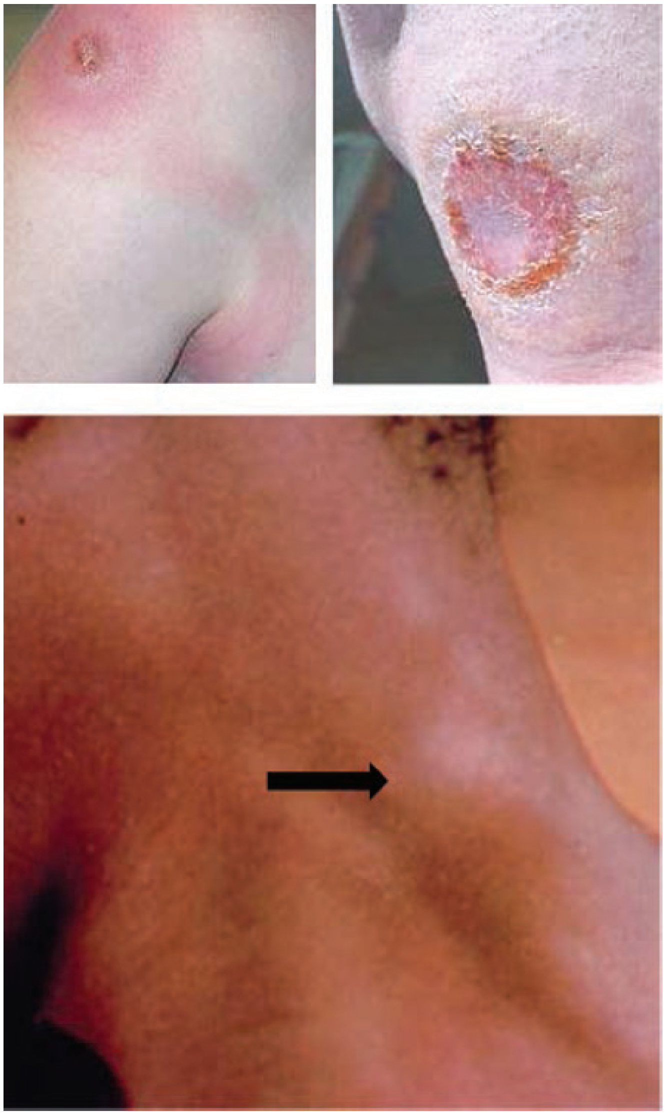
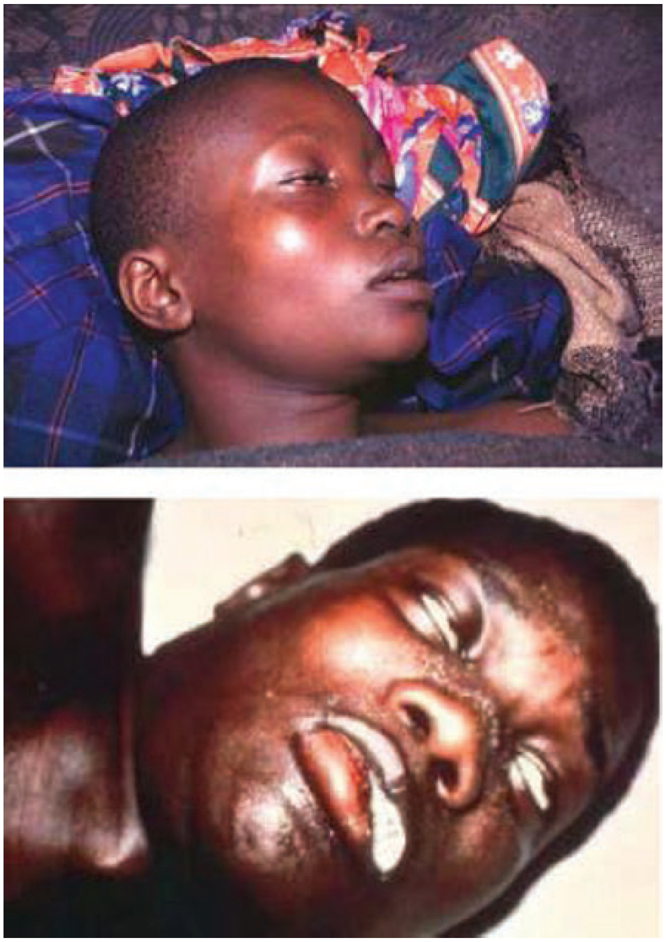


Figure 28.7 (Upper, left) Trypanosomal chancre on shoulder of patient, with lymphangitis toward axilla; (right) trypanosomal chancre on throat of patient (courtesy of CDC Public Health Image Library, Peter L. Chiodini, Hospital for Tropical Diseases, London). (Lower) Winterbottom's sign (enlarged cervical lymph nodes in the posterior cervical triangle; arrow) (Armed Forces Institute of Pathology photograph). doi:10.1128/9781555819002.ch28.f7

polyclonal B-cell activation with a marked increase in the levels of IgM, heterophile and anti-DNA antibodies, rheumatoid factor, and circulating immune complex in serum. The sustained high IgM levels are a result of the parasite producing variable antigen types which allow the organism to evade the patient's defense system. *In an immunocompetent host, the absence of elevated IgM levels in serum rules out trypanosomiasis.*

Stage II. Once trypomastigotes invade the CNS, the sleeping sickness stage of the infection is initiated (stage II disease). The trypomastigotes are found primarily in the frontal lobes, pons, and medulla. Behavioral and personality changes are seen during CNS invasion. Gambian trypanosomiasis is characterized by steady progressive meningoencephalitis, apathy, confusion, fatigue, coordination loss, and somnolence (Fig. 28.8). In the terminal phase of the disease, the patient becomes emaciated and progresses to profound coma and death, usually from secondary infection. Although this disease has always been considered as invariably fatal, it is now well documented that this outcome may not necessarily be the case. Information is found in a long-term follow-up (15 years) of a cohort of 50 human African trypanosomiasis patients from the Ivory Coast among whom 11 refused treatment after their initial diagnosis (9). In 10 out of 11 subjects who continued to refuse treatment despite repeated visits, parasite clearance was observed using both microscopy and PCR. Most of

Figure 28.8 Comatose patients with African sleeping sickness (courtesy of WHO). doi:10.1128/9781555819002.ch28.f8



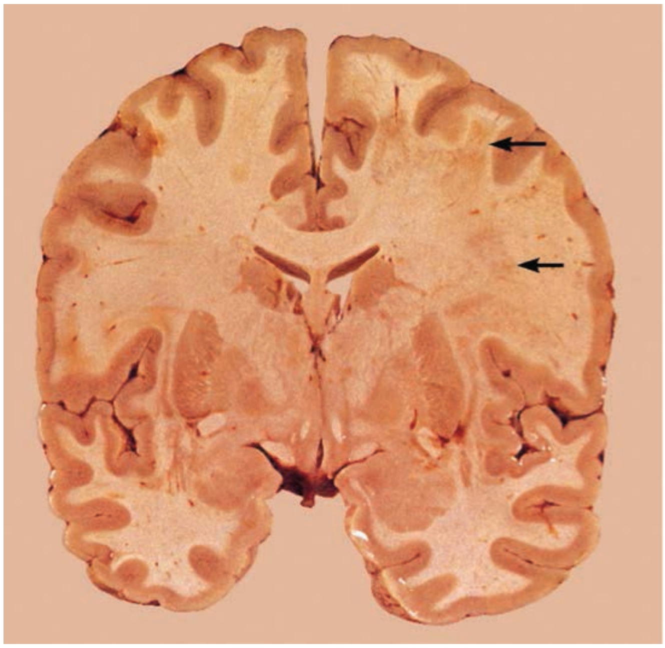
these subjects (7/10) also displayed decreasing serological responses, becoming progressively negative to trypanosome variable antigens.

Immunosuppression in patients with Gambian trypanosomiasis is manifested by decreases in both cellular and humoral immunity. Apparently, increased prostaglandin D2 may in part account for the increased somnolence and the immunosuppression within the CNS. Measurement of these levels may provide confirmation for CNS involvement (10).

With invasion of the CNS (Fig. 28.9), there is a progressive leptomeningitis, and CSF findings include increased protein levels, elevated IgM levels, lymphocytosis, and morular cells of Mott (Fig. 28.10). Morular (mulberry) cells are altered plasma cells whose cytoplasm is filled with proteinaceous droplets. Morular cells are not seen in all patients; however, they are characteristic of African trypanosomiasis. The spherules of morular cells are not metachromatic with toluidine blue at pH 8, and most spherules stain yellow with phosphotungstic acid-hematoxylin stain. This distinguishes them from globule leukocytes. They differ from Russell bodies of plasma cells, which are Gram positive. Russell bodies are eosinophilic, large, homogeneous immunoglobulin-containing inclusions usually found in a plasma cell undergoing excessive synthesis of immunoglobulin.

In humans with African trypanosomiasis, autoantibodies have been found, including autoantibodies directed to RBCs, smooth muscle, liver and cardiolipid nucleic acids (DNA and RNA), and intermediate filaments of the cytoskeleton in smooth muscle cells. Autoantibodies to components of CNS myelin have also been found, as have those to neurofilament proteins in sera and CSF from untreated patients with stage II disease and CNS involvement (11). The CSF antibodies to neurofilament proteins were IgM in 22 of 25 patients and IgG in 8 of 25.

Figure 28.9 Coronal section of brain showing congested white matter and petechial hemorrhages (arrows) (Armed Forces Institute of Pathology photograph).
doi:10.1128/9781555819002.ch28.f9



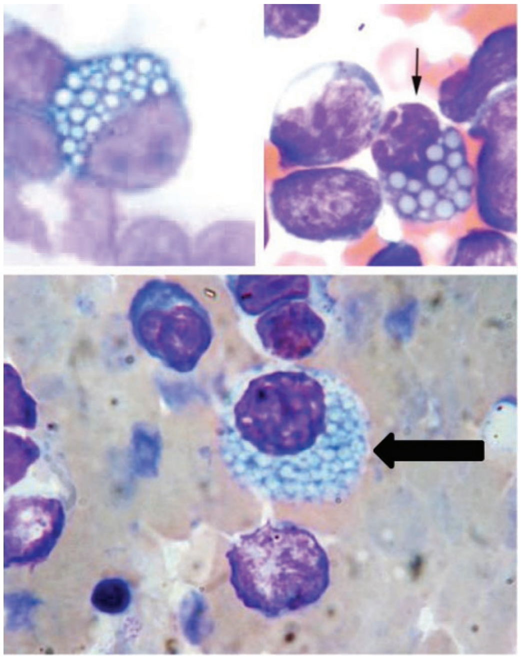


Figure 28.10 Morular (Mott) cells in the CSF (arrows), associated with trypanosomiasis. (Top left image courtesy of the Federation of Infectious Diseases Societies of Southern Africa. Top right image image from Bavle RM, J Oral Maxillofac Pathol, **17**:2–3, 2013. Bottom image courtesy of Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney Medical School, Sydney, Australia.)
doi:10.1128/9781555819002.ch28.f10

Diagnosis

Physical findings and clinical history are very important in establishing the diagnosis. Diagnostic symptoms include irregular fever, enlargement of the lymph nodes (particularly those of the posterior triangle of the neck [Winterbottom's sign]), delayed sensation to pain (Kerandel's sign), and erythematous skin rashes. However, diagnosing imported human African trypanosomiasis outside areas of endemicity is difficult and diagnosis is often delayed. A recent case was reported with *T. gambiense* with an unusually long incubation period of at least 7 years (12). Definitive diagnosis depends on demonstration of trypomastigotes in blood, lymph node aspirates, sternum bone marrow, and CSF. Due to the greater frequency of high parasitemia, there is a better chance of detecting organisms in body fluids in infections caused by *T. brucei rhodesiense* than in those caused by *T. brucei gambiense*. Because of periodicity, parasite numbers in the blood may vary; therefore, multiple specimens should be collected and a number of techniques should be used to detect the trypomastigotes (Fig. 28.4 and 28.6; Tables 28.2 and 28.3). Health care personnel must adhere to standard precautions when handling specimens from patients with suspected cases of African trypanosomiasis because the trypomastigotes are highly infectious.

Chancre Aspirate. Organisms can be obtained from the chancre by using puncture techniques; the fluid is then examined as wet mounts and stained smears using any of the blood stains. This approach is seldom used; patients are generally not seen until after the chancre has already healed.

Lymph Node Aspirate. When enlarged cervical lymph nodes are seen, they can be punctured and the aspirate can be examined as a wet preparation for the presence of motile trypomastigotes. The aspirated material can also be stained using Giemsa or other blood stains. Depending on a number of factors, the sensitivity ranges from about 40 to 80% (2).

Blood. Blood can be collected from either finger stick or venipuncture. Venous blood should be collected in a tube containing EDTA (fill the tube to obtain correct anticoagulant/blood ratio). Multiple slides should be made for examination, and multiple blood examinations should be done before trypanosomiasis is ruled out. Parasites are found in large numbers in the blood during the febrile period and in small numbers when the patient is afebrile. In addition to thin and thick blood films, a buffy coat concentration method is recommended to detect the parasites. Parasites can be detected on thin blood films with a detection limit at approximately 1 parasite/200 microscopic fields (high dry power magnification, ×400); they can be detected on thick blood smears when the numbers are greater than 2,000/ml and when they are greater than 100/ml with hematocrit capillary tube concentration.

TABLE 28.2 Characteristics of East and West African trypanosomiasis

Characteristic	East African	West African
Organism	*Trypanosoma brucei rhodesiense*	*Trypanosoma brucei gambiense*
Vector	Tsetse fly, *Glossina morsitans* group	Tsetse fly, *Glossina palpalis* group
Primary reservoirs	Animals (wild and domestic)	Humans
Illness	Acute (early CNS invasion), <9 mo	Chronic (late CNS invasion), months to years
Lymphadenopathy	Minimal	Prominent
Parasitemia	High	Low
Epidemiology	Anthropozoonosis, game parks	Anthroponosis, rural populations
Diagnostic stage	Trypomastigote	Trypomastigote
Recommended specimens	Chancre aspirate, lymph node aspirate, blood, CSF	Chancre aspirate, lymph node aspirate, blood, CSF
Areas of high endemicity[a]	Western Africa	Western Africa
	None	Côte d'Ivoire
		Guinea
	Northeastern Africa	Northeastern Africa
	Ethiopia (endemicity unknown)	Chad
		Sudan (epidemics)
	Central Africa	Central Africa
	Democratic Republic of the Congo (epidemics)	Democratic Republic of the Congo (epidemics)
	United Republic of Tanzania	United Republic of Tanzania
	Uganda	Rwanda (endemicity unknown)
	Rwanda (endemicity unknown)	Cameroon
		Central African Republic
		Congo
	Southern Africa	Southern Africa
	Angola (epidemics)	Angola (epidemics)
	Namibia (unknown)	Namibia (unknown)
	Mozambique	

[a] Other African countries are also involved, but with low levels of endemicity. For a more complete list, see reference 3.

TABLE 28.3 Methods available for the diagnosis of African trypanosomiasis (*T. brucei gambiense*)

Method	Description	Comments
Chancre aspirate	Puncture; fluid obtained is examined as wet preparation and Giemsa-stained smears (other blood stains are acceptable).	Not commonly used; patients are often seen after the chancre has already healed.
Lymph node aspirate	Puncture; fluid obtained is examined as wet preparation and Giemsa-stained smears (other blood stains are acceptable).	Sensitivity varies between 40 and 80%; yield is very low with seronegative patients. Not recommended unless clinical signs are present.
Blood	Both wet films and stained thick and thin blood films can be examined.	Organism motility among red blood cells enhances detection; sensitivity about 1 organism per 200 microscopic fields (~10,000 trypomastigotes/ml) (using high dry objective). Stained thick blood film improves sensitivity with detection threshold of ~5,000 trypomastigotes/ml. Organism morphology better on thin blood film. Giemsa or other blood stains are acceptable.
Microhematocrit centrifugation	Centrifugation of capillary tubes containing anticoagulant filled with finger stick blood (end of tube sealed with plastic clay); tube examined under microscope, and organisms are concentrated at buffy coat layer	
Microhematocrit centrifugation (QBC)	High-speed centrifugation of blood in capillary tubes containing acridine orange; motile organisms identified by fluorescent nuclei and kinetoplasts in the buffy coat layer	The technique is used sporadically within the United States (cost is a factor).
Mini-anion-exchange centrifugation	Separation of trypomastigotes from venous blood using anion-exchange chromatography; subsequent concentration at tip of glass tube	Sensitivity of <100 organisms/ml; procedure is very time-consuming. The columns are produced in Belgium. Method validation is under way.
Cerebrospinal fluid	White blood cell count used to determine disease stage; cell-counting chambers should have a capacity of 1 µl (Fuchs-Rosenthal and Neubauer chambers); do not dilute CSF	Patients (trypomastigotes in lymph node or blood) with >20 cells/µl of CSF are treated as having second-stage disease; therapeutic approach depends on the geographic areas and drug selection. Trypomastigotes in CSF are confirmatory for second-stage disease; sensitivity is increased by centrifugation.
Antigen detection	A simple and rapid test, the card indirect agglutination trypanosomiasis test (TrypTect CIATT), is available, primarily in areas of endemic infection; an ELISA method has been used to detect antigen in serum and CSF.	Good sensitivity but possible specificity issues with CIATT; ELISA antigen is rapidly cleared after therapy.
Antibody detection	CATT fast/simple agglutination assay for *T. brucei gambiense*-specific antibodies; latex agglutination is another option; immunofluorescence excellent (sensitivity and specificity), but lower with filter paper eluates; ELISA available for serum, filter paper eluates, and CSF	Sensitivity reported on whole blood (87–98%); negative predictive value is excellent for mass screening. Both false positives and false negatives have been reported. Can be performed with blood-impregnated filter paper. Latex agglutination shows higher specificity but lower sensitivity than fluorescence. Latex is also more specific than CATT on diluted specimens.
Molecular methods	A number of methods have been reported; however, cost and technical issues have prevented their use in the routine clinical laboratory.	Some methods appear to be very sensitive; simplification of the method may lead to methods adaptable to routine clinical laboratory use. There have been a number of recently developed tests for molecular diagnosis of sleeping sickness (30).

Microhematocrit Centrifugation. The original microhematocrit centrifugation technique uses capillary tubes containing anticoagulant, filled with finger stick blood. The bottom of the tube is sealed with plasticine, and the tube is centrifuged in a hematocrit centrifuge for about 8 min. The trypomastigotes are concentrated at the buffy coat layer. The tubes are then placed in a special holder and examined through the glass at low magnification for motile organisms (13).

Microhematocrit centrifugation with use of the QBC malaria tube (glass capillary tube and closely fitting plastic insert; CBC malaria blood tubes [QBC Diagnostics, Inc., Port Matilda, PA]) has been used for the detection of blood parasites. At the end of centrifugation of 50 to 60 µl of capillary or venous blood (5 min in a QBC centrifuge; 14,387 × *g*), parasites or erythrocytes containing parasites are concentrated into a small, 1- to 2-mm region near the top of the RBC column and are held close to the wall of the tube by the plastic float, thereby making them readily visible by microscopy. Tubes precoated with acridine orange provide a stain which induces fluorescence in the parasites. This method automatically prepares a concentrated smear, which represents the distance between the float and the walls of the tube. Once the tube is placed into the plastic holder (Paraviewer) and immersion oil is applied onto the top of the hematocrit tube (no coverslip is necessary), the tube is examined with a 40× to 60× oil immersion objective (there must be a working distance of 0.3 mm or greater) (refer to chapter 7).

The range of sensitivity for the QBC test has been as low as 55% up to >90% compared with microscopy. While most think the method is rapid, some think there are some disadvantages such as (i) the high cost of capillary tubes and equipment, (ii) problems in species identification and quantification, and (iii) technical problems, broken capillaries, and the fact that the capillaries cannot be stored for later reference. This may not be the most appropriate approach for a laboratory that receives a small number of specimens for blood parasite testing, but it could be helpful in other settings.

Mini-Anion-Exchange Centrifugation. The mini-anion-exchange method allows the separation of trypomastigotes from venous blood by anion-exchange chromatography, with subsequent low-speed centrifugation into the tip of the glass tube. The tube is then examined under the microscope with a sensitivity of <100 organisms/ml. To increase the sensitivity of this test, 350 µl of buffy coat withdrawn from 5 ml of blood is used instead of blood (14).

Cerebrospinal Fluid. If CSF is examined, a volume greater than 1 ml, preferably 5 ml or more, should be collected. CSF examination must be conducted with centrifuged sediments. The presence of Mott cells in the CSF is pathognomonic for trypanosomiasis (Fig. 28.10). Mott cells are rarely observed when there are few white blood cells in the CSF. When trypomastigotes are present in undetectable numbers in the blood, they may be seen in aspirates of inflamed lymph nodes; however, attempts to demonstrate them histopathologically are not practical. Blood and CSF specimens should be examined during therapy to evaluate the response and again 1 to 2 months after therapy. Patients treated for CNS disease should be monitored clinically for 2 to 3 years after completion of therapy to detect a relapse.

Antigen Detection. An enzyme-linked immunosorbent assay (ELISA) method has been used to detect antigen in serum and CSF (15). It was noted that antigen was rapidly cleared from the circulation following successful therapy. This method could also be used for clinical staging of disease to determine whether there was CNS infection and for follow-up to therapy.

A simple and rapid test, the card indirect agglutination trypanosomiasis test (TrypTect CIATT), is available, primarily in areas of endemic infection, for the detection of circulating antigens in persons with African trypanosomiasis. The test is normally performed on a drop of freshly collected heparinized blood and is followed by a more specific confirmation test on diluted blood, plasma, or serum. The sensitivity of the test (95.8% for *T. brucei gambiense* and 97.7% for *T. brucei rhodesiense*) is significantly higher than that of lymph node puncture, microhematocrit centrifugation, and CSF examination after single and double centrifugation (16). Its specificity is excellent, and it has a high positive predictive value. A complement-mediated prozone phenomenon may occur, causing lower test sensitivity at lower sample dilutions. A simple solution is the addition of a Ca^{2+} chelating agent such as EDTA to the diluted blood, plasma, or serum (17).

Using a panel of 80 randomized *T. brucei gambiense* infection and control sera, a lateral flow diagnostic device prototype showed reasonable sensitivity (88%) and specificity (93%) using visual readout in detecting *T. brucei gambiense* infections (18).

Antibody Detection. The card agglutination test for trypanosomiasis (CATT/*T. brucei gambiense*) is more sensitive than methods that require visualization of the trypomastigotes with clinical specimens and can be performed with blood, plasma, or serum. The test is produced by the Institute of Tropical Medicine in Antwerp, Belgium, and is widely used for mass population screening. This approach can also be used with blood-impregnated filter paper. It has been recommended that treatment with pentamidine should be considered for all serologically suspected individuals with a CATT-plasma end dilution titer of ≥1:16 in areas of moderate to high disease prevalence (19).

A semiquantitative ELISA, using VSG of *T. brucei gambiense* as the antigen, has been developed for the detection of antibodies of different immunoglobulin isotypes in serum and CSF of patients with sleeping sickness. In serum and CSF, a drastic increase in the level of IgG (basically IgG1) as well as in the IgM level was seen; an increase in the IgG3 level was also seen, while the IgA level remained relatively normal. Measurement of immunoglobulin and trypanosome-specific antibody concentrations in serum and CSF allows calculation of intrathecal antibody synthesis and may be a possible tool for the determination of clinical stages of sleeping sickness (20). Both latex agglutination and immunofluorescence tests are also available; however, sensitivity results have not been as good with the latex approach as with fluorescence. Specificity with the latex test appears to be better than the CATT with diluted blood; use of the latex test also decreased the workload and costs (21, 22).

Using ELISA, anti-trypanosome-derived lymphocyte triggering factor (TLTF) antibodies were detected in the serum of all African trypanosomiasis patients, the antibody levels being independent of clinical stage of the disease. This indicates that TLTF is released by *T. brucei gambiense* bloodstream forms early on in the infection in sufficient amounts to induce a humoral immune response. In the CSF samples, antibodies were only detectable in patients with evidence of trypanosome invasion of the CNS, suggesting TLTF release by intrathecally resident parasites and subsequent intrathecal antibody production (23).

Serologic techniques which have been widely used for epidemiologic screening include indirect fluorescent-antibody assays, ELISA, the indirect hemagglutination test, and the card agglutination trypanosomiasis test (see above). A major serodiagnostic problem in areas where the disease is endemic is that many in the population have elevated antibody levels due to exposure to animal trypanosomes that are noninfectious to humans. Serum and CSF IgM concentrations are of diagnostic value. CSF antibody titers should be interpreted with caution because of the lack of reference values and the possibility that the CSF contains serum as the result of a traumatic tap.

Molecular Methods. Accurate identification of trypanosome species is necessary in order to clarify the epidemiology of human and animal African trypanosomiasis, but this has proved difficult. Great progress has been made through the application of DNA probe technology, although this has also revealed greater complexity than was previously supposed (24). *T. brucei* organisms have been detected in human blood by using a nonradioactive branched-DNA-based method. Buffy coat specimens are used, and the results agree with those obtained using buffy coat microscopy. The assay can be performed with crude lysates without the need for extensive sample preparation. Also, because it amplifies the signal from a target molecule rather than the target itself, this technique is not prone to the artifact problems that can be seen with PCR-based tests (25). The development of a peptide nucleic acid fluorescence *in situ* hybridization probe appears to offer an excellent diagnostic tool. By combining this test with a cytospin step, the limit of detection from blood can be improved to 5 parasites/ml, a level of sensitivity that equals an optimal PCR detection limit with blood specimens (26). A newer PCR technique targets the gene encoding the small ribosomal subunit in order to identify and differentiate all clinically important African trypanosome species and some subspecies. This method appears to be more economical, simple, and sensitive than some other screening methods, and it yields more detailed information (27). Other real-time PCR options appear to be suitable for use within the routine clinical laboratory (28). The loop-mediated isothermal amplification (LAMP) reaction is a method that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions with only simple incubators. An added advantage of LAMP over PCR-based methods is that DNA amplification can be monitored spectrophotometrically and/or with the naked eye without the use of dyes (29, 30).

Animal Inoculation and Culture. *T. brucei gambiense* isolation in small laboratory animals is usually unsuccessful; in contrast, *T. brucei rhodesiense* readily infects animals. Cultivation is not practical for most diagnostic laboratories but is more successful than animal inoculation.

KEY POINTS—LABORATORY DIAGNOSIS

Trypanosoma brucei gambiense

1. Trypomastigotes are highly infectious, and health care workers must use blood-borne-pathogen precautions.
2. Trypomastigotes may be detected in aspirates of the chancre and enlarged lymph nodes in addition to blood and CSF.
3. Concentration techniques, such as centrifugation of CSF and blood, should be used in addition to thin and thick smears (Giemsa, Wright's, or other blood stains).
4. Trypomastigotes are present in largest numbers in the blood during febrile periods.
5. Examinations of multiple daily blood samples may be necessary to detect the parasite.
6. Blood and CSF specimens should be examined during therapy and again 1 to 2 months posttherapy.

Treatment

All drugs currently used in the therapy of African trypanosomiasis are toxic and require prolonged administration. Treatment should be started as soon as possible and is based on the patient's symptoms and laboratory findings. The choice of antiparasitic drug depends on whether the CNS is infected. Suramin or pentamidine isethionate can be used when the CNS is not infected. Pentamidine is currently the drug of choice for the hemolymphatic stage, with suramin being an alternative; it appears that in early CNS involvement, the results of pentamidine (94% cure rate in a group of 52 patients observed for 2 years) are comparable to those obtained with melarsoprol or eflornithine in terms of tolerance and availability (31, 32). Pentamidine isethionate does not cross the blood-brain barrier and is administered intramuscularly; side effects include an immediate hypotensive reaction, nausea, vomiting, and Herxheimer-type (inflammatory) reactions. Suramin (Bayer 205), a diamidine, is effective in treating the hemolymphatic stage and CNS disease of *T. brucei gambiense*. When suramin is used, a test dose should be given to the patient to ensure that the drug can be tolerated. It should not be given to patients with renal disease, and if protein, RBCs, or casts are detected in the urine, treatment should be stopped. Suramin is given intravenously; its side effects include nausea, vomiting, loss of consciousness, seizures, pruritus, edema, and hepatitis. Although rare, fatalities have been reported during suramin therapy. Suramin can be obtained under an Investigational New Drug (IND) protocol from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, GA, 30333; 404-639-3670 (evenings, weekends, or holidays: 404-639-2888) (32).

Melarsoprol, a toxic trivalent arsenic derivative, is effective for both hemolymphatic and neural stages but is recommended for treatment of late-stage sleeping sickness. It is administered intravenously, and its side effects include nausea, vomiting, encephalopathy, and exfoliative dermatitis. Melarsoprol has been used for CNS involvement for years; however, relapse occurs in up to 6% of patients. Arsenic-related encephalopathy occurs in 10% of patients, and prednisolone treatment may help reduce the incidence. The mortality due to this side effect depends on whether supportive treatment is given, but it is often above 50% (33). Resistance of both *T. brucei gambiense* and *T. brucei rhodesiense* to melarsoprol has been noted.

Eflornithine (DL-α-difluoromethylornithine; DFMO) has been used for more than 10 years to treat melarsoprol-resistant *T. brucei gambiense* infection with or without CNS involvement; treatment is started intravenously and is followed by oral therapy. In one study, 47 patients with a relapse following a first treatment were treated with a 7-day course of intravenous eflornithine (100 mg/kg every 6 h) and monitored for 2 years; the failure rate was 6.5% (34). This approach appears to provide adequate treatment in cases of Gambian trypanosomiasis relapsing after treatment with another drug. Disadvantages of eflornithine therapy include the prolonged course of treatment and frequent side effects of diarrhea and anemia. With adequate treatment, non-CNS disease has a cure rate of >80%. All treated patients should be followed up twice yearly for at least 2 to 3 years. Specific drug dosage information is provided in chapter 36; however, apparently eflornithine is no longer commercially available but is available in limited supply from the World Health Organization (32).

Nifurtimox (Lampit), a nitrofuran, has been used to treat patients infected with *T. brucei gambiense* who do not respond to melarsoprol. The relapse rate is quite high when nifurtimox is used alone, but the drug is quite effective when used in combination with eflornithine (35). It is given orally. Side effects include confusion, tremor, vertigo, anorexia, and weight loss. This drug is not recommended for the treatment of *T. brucei rhodesiense* infections. Nifurtimox can be obtained under an IND protocol from the CDC Drug Service (32).

Epidemiology and Prevention

T. brucei gambiense is transmitted from person to person by the bite of the tsetse fly (*G. palpalis* and *G. tachinoides*) after infectivity develops within the insect. The development cycle in the fly, depending on temperature and moisture, varies from 12 to 30 days and averages 20 days. Fewer than 10% of tsetse flies become infective after obtaining blood from infected patients, and vertical transmission from infected fly to offspring is not known to occur. *G. palpalis* and *G. tachinoides* can be found in areas of thick shrubbery and trees near the banks of rivers, streams, or water holes; therefore, transmission readily occurs when people frequent these areas. Both female and male tsetse flies can transmit the infection. In addition to biological transmission, the tsetse fly may mechanically transmit the infection with its proboscis if it bites an uninfected person within a few hours of biting an infected person. Congenital transmission in humans has also been documented.

Although *T. brucei gambiense* can be transmitted to animals, no animal reservoirs for West African sleeping sickness have been documented. Trypanosomal strains isolated from hartebeest, kob, chickens, dogs, cows, and domestic pigs in West Africa are identical to those isolated from humans in the same area. These findings suggest that animals may serve as reservoirs; however, there is no indication that these animal trypanosomal forms are directly transmitted to humans and that a patent infection will develop. Epidemiologic evidence suggests that in areas of endemic infection, transmission may be entirely from human to human. Asymptomatic individuals are thought to be the residual reservoir of the disease.

In areas where West African sleeping sickness is endemic, *T. brucei gambiense* has been controlled by eliminating the parasite through regular population-screening programs. This approach effectively reduces the prevalence of the disease to low levels; however, with interruptions in surveillance, resurgence of the disease will occur. An example of resurgence of West African trypanosomiasis was well documented in southern Sudan, where the disease prevalence found was among the highest ever documented.

Although game parks in Tanzania (Tarangire and Serengeti National Parks) were thought to be low-risk areas for African trypanosomiasis, during 2001 nine patients from these areas were diagnosed with *T. brucei gambiense* infection. All of the South African patients but one were European nationals. During their trip or shortly after their return, the patients, all febrile, were seen by general practitioners or emergency departments. Most patients were seen during the primary disease stage; however, several showed cerebral manifestations of the secondary stage. Most patients also showed a typical skin lesion, the trypanosome chancre. Diagnosis was established by examining thin and thick blood films. Although three patients had multiorgan failure, and specific medication was difficult to obtain, drug treatment proved successful in all but one patient, who died (36).

At present, there are no areas where *T. brucei gambiense* and *T. brucei rhodesiense* are endemic together. Although foci of both organisms exist in Uganda, they appear to be geographically separate. Based on evidence of the spread of cattle carrying *T. brucei rhodesiense* further north, a merger of the two foci could create tremendous problems since the diagnosis and treatment of the two diseases differ significantly (37).

Vector control measures have met with limited success. Methods have included clearing streams of underbrush, eliminating breeding grounds, using insecticides, using fly traps, and releasing sterile male flies. Because tsetse flies have a low reproductive rate and have not developed insecticide resistance, they are very tempting control targets. The most effective control measures include an integrated approach to reduce the human reservoir of infection and to use insecticides and fly traps.

In regions where the disease is endemic, natives appear to be more resistant to infection than are new arrivals to the area, even though there is no evidence of acquired immunity. West African sleeping sickness affects primarily the rural populations, and tourists are rarely infected. Chemoprophylaxis is not recommended because of drug toxicity, and vaccines are unavailable. It may be possible to develop a vaccine, because immunity to reinfection occurs with *T. brucei gambiense*. However, some believe that the almost unlimited capacity for antigenic variation of surface glycoproteins by the African trypanosomes may be the major hurdle for producing a vaccine (7). Others feel there may be additional factors required to induce Th1 memory cells specific for common antigens that enhance innate resistance.

Tourists are usually not at great risk unless they are traveling and spending long periods in rural areas of western and central Africa. Persons visiting areas where the disease is endemic should avoid tsetse fly bites by wearing protective clothing (long-sleeved shirts and long pants); khaki or olive-colored clothing is optimal, since the tsetse fly is attracted to bright colors and very dark colors. Because heavy clothing is not always practical owing to heat and humidity, other measures, including the use of insect repellents, bed netting, and screens, are recommended. It has also been recommended not to ride in the back of jeeps, pickup trucks, or other open vehicles; the tsetse fly is attracted to the dust created by moving vehicles and wild animals. Bushes should be avoided, since the tsetse fly is less active during the hot period of the day and rests in bushes but will bite if disturbed.

Trypanosoma brucei rhodesiense

T. brucei rhodesiense is closely related to *T. brucei gambiense*, and the two subspecies are morphologically indistinguishable. Stephans and Fantham in 1910 discovered *T. brucei rhodesiense* in the blood of a patient in Rhodesia with symptoms consistent with "sleeping sickness." In 1912, this organism was shown to be transmitted by a different species of tsetse fly (*Glossina morsitans*) from that associated with *T. brucei gambiense* (*G. palpalis*). *T. brucei rhodesiense* was also differentiated from *T. brucei gambiense* on the basis of virulence in rats and detection of a morphologic variant not previously found in *T. brucei gambiense*. Many investigators think that *T. brucei rhodesiense* is the human strain of *T. brucei*, a common trypanosome that is found in many game animals and causes a fatal disease known as nagana in cattle. In 1961, Ormerod indicated that the original strains of *T. brucei rhodesiense* probably arose in the Zambesi basins as mutants from *T. brucei* (38). In the early 1900s, human volunteer inoculation studies with *T. brucei* resulted in no human infections. Currently, it is accepted that there are two subspecies within the *T. brucei* species, and they have been designated *T. brucei gambiense* and *T. brucei rhodesiense*. *T. brucei rhodesiense* (which causes Rhodesian trypanosomiasis or East African sleeping sickness) is more limited in distribution than *T. brucei gambiense*, being found only in central East Africa, where the disease has been responsible for some of the most serious obstacles to economic and social development in Africa. Within this area, the vast majority of tsetse flies prefer animal blood, which therefore limits the raising of livestock. The infection has a greater morbidity and mortality than does *T. brucei gambiense* infection, and game animals, such as the bushbuck, and cattle are natural reservoir hosts. Biochemical evidence shows that the human-infective *T. brucei rhodesiense* differs from the human-infective *T. brucei brucei* because of its resistance to lysis by components in human serum. This resistance can be acquired by sensitive strains and is caused by the decreased internalization of a trypanolytic factor. Thus, *T. brucei rhodesiense* appears to be a host range variant of *T. brucei brucei* (2). While human- and nonhuman-infective strains are genetically closely related, very little gene flow appears to be occurring between these two host range variants in natural populations. Restricted gene flow could be due to a lack of genetic exchange caused by lack of opportunity or some type of biological barrier. Evidence also suggests that genetic exchange can occur in the tsetse fly; however, large-scale studies of *T. brucei* genotypes in tsetse flies are difficult, especially since only 0.1% of the flies are usually infected (2).

Life Cycle and Morphology

The life cycle and reproduction of *T. brucei rhodesiense* are similar to those of *T. brucei gambiense* (Fig. 28.4). The tsetse fly vectors for transmission of *T. brucei rhodesiense* are *G. pallidipes*, *G. morsitans*, and *Glossina tachinoides*. The morphology of *T. brucei rhodesiense* in tsetse flies and humans is similar to that of *T. brucei gambiense*, except that when the parasite is inoculated into laboratory animals, the posterior nucleate forms are more common.

Clinical Disease

T. brucei rhodesiense produces a more rapid, fulminating disease than does *T. brucei gambiense*. Fever, severe headaches, irritability, extreme fatigue, swollen lymph nodes, and aching muscles and joints are common symptoms. Progressive confusion, personality changes, slurred speech, seizures, and difficulty in walking and talking occur as the organisms invade the CNS. The early stages of the pathologic process parallel those of *T. brucei gambiense* infections; however, the disease progresses more rapidly, such that death may occur before there is extensive CNS involvement even though CNS invasion occurs early. The incubation period is short, often within 1 to 4 weeks, with trypomastigotes being more numerous and appearing earlier in the blood. Lymph node involvement is less pronounced, and Winterbottom's sign may be absent. Febrile paroxysms are more frequent, and the patients are more anemic and more likely to develop myocarditis or jaundice. Some patients may develop persistent tachycardia, and death may result from arrhythmia and congestive heart failure due to pancarditis. Myocarditis may develop in patients with Gambian trypanosomiasis but is more common and severe with the Rhodesian form. Review of a splenectomized patient with Rhodesian trypanosomiasis and CNS involvement revealed that the onset of symptoms, laboratory studies, and disease progression did not differ from those for reported cases in healthy individuals (39). However, the role of the spleen in trypanosomiasis is not well understood.

Diagnosis

The techniques used are similar to those for detecting Gambian trypanosomiasis; however, trypomastigotes are more numerous in the blood in the Rhodesian form (Table 28.2). A differential diagnosis may be possible on geographic grounds, but there are areas where the two forms overlap. Because infection with *T. brucei rhodesiense* is so fulminant, the immune response with humoral antibody will be weaker than that for *T. brucei gambiense* infections; therefore, serodiagnosis may be difficult. Blood and CSF specimens should be examined during and 1 to 2 months after therapy. Unfortunately, the lack of progress in diagnostic methods is often related to the many obstacles encountered in the modification of basic research to field-applicable diagnostics.

T. brucei rhodesiense is more adaptable to cultivation than is *T. brucei gambiense*. A number of media have been described; however, cultivation is not a practical diagnostic approach. See Algorithm 28.1, Table 28.4, and the "Key Points—Laboratory Diagnosis" presented below.

KEY POINTS—LABORATORY DIAGNOSIS

Trypanosoma brucei rhodesiense

1. Trypomastigotes are highly infectious, and health care workers must use blood-borne-pathogen precautions.
2. Trypomastigotes may be detected in aspirates of the chancre and enlarged lymph nodes in addition to blood and CSF; trypomastigotes tend to be more numerous in the blood in Rhodesian trypanosomiasis, and diagnosis is normally confirmed from blood examination.
3. Concentration techniques, such as centrifugation of CSF and blood, should be used in addition to thin and thick smears (Giemsa, Wright's, or other blood stains). However, concentration methods may not be required, since organisms tend to be numerous in the blood.
4. Trypomastigotes are present in the blood in largest numbers during febrile periods.
5. Examinations of multiple daily blood samples may be necessary to detect the parasite.
6. Blood and CSF specimens should be examined during therapy and 1 to 2 months posttherapy.
7. Culture is possible using well-described media but is probably not practical for most diagnostic laboratories; again, the use of blood-borne-pathogen precautions is mandatory.

Treatment

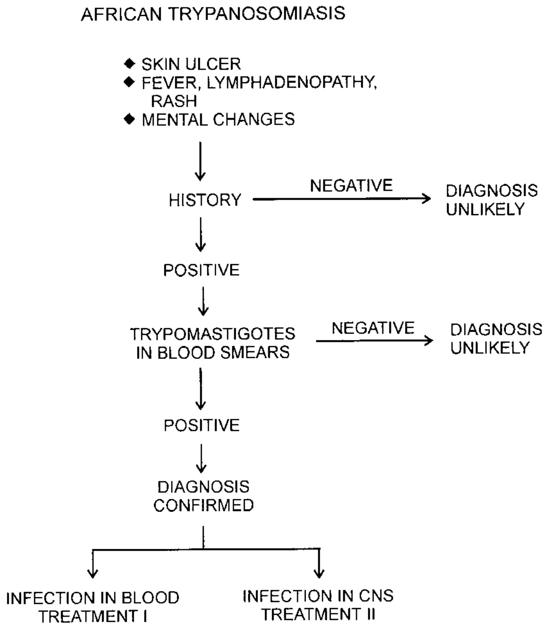
Because East African trypanosomiasis is such a serious illness, treatment should be started as soon as possible and is based on the patient's symptoms and laboratory findings. Within the United States, medication and therapeutic advice is available through the CDC. Without treatment, nearly all patients develop neural involvement and die; however, in treated patients, cure rates are above 80%, even in those with CNS infection. The choice of antiparasitic drug depends on whether the CNS is infected; usually patients are hospitalized during treatment. Suramin can be used early in the course of the infection, when the CNS is not infected.

TABLE 28.4 Methods available for the diagnosis of African trypanosomiasis (*T. brucei rhodesiense*)

Method	Description	Comments
Chancre aspirate	Puncture; fluid obtained is examined as wet preparation and Giemsa-stained smears (other blood stains are acceptable).	Not commonly used; patients are often seen after the chancre has already healed.
Lymph node aspirate	Puncture; fluid obtained is examined as wet preparation and Giemsa-stained smears (other blood stains are acceptable).	Normally not required, disease presents as acute febrile illness.
Blood	Both wet films and stained thick and thin blood films can be examined.	Organism motility among red blood cells enhances detection; trypomastigotes are generally numerous. Organism morphology better on thin blood film. Giemsa or other blood stains acceptable.
Microhematocrit centrifugation	Centrifugation of capillary tubes containing anticoagulant filled with finger stick blood (end of tube sealed with plastic clay); tube is examined under microscope; organisms are concentrated at buffy coat layer.	Simple method, widely used for epidemiology studies; based on large numbers of organisms in *T. brucei rhodesiense* infections. Buffy coat should be highly motile with organisms.
Microhematocrit centrifugation (QBC)	High-speed centrifugation of blood in capillary tubes containing acridine orange; motile organisms identified by fluorescent nuclei and kinetoplasts in the buffy coat layer.	The technique is used sporadically within the United States (cost is a factor).
Mini-anion-exchange centrifugation	Separation of trypomastigotes from venous blood using anion-exchange chromatography; subsequent concentration at tip of glass tube	Sensitivity of <100 organisms/ml; procedure is very time-consuming. The columns are produced in Belgium. Method validation is under way. Not required for diagnosis of *T. brucei rhodesiense* (many organisms present).
Cerebrospinal fluid	White blood cell count used to determine disease stage; cell-counting chambers should have a capacity of 1 µl (Fuchs-Rosenthal and Neubauer chambers); do not dilute CSF.	Patients (trypomastigotes in lymph node or blood) with >20 cells/µl of CSF are treated as having second-stage disease; therapeutic approach depends on the geographic areas and drug selection. Trypomastigotes in CSF are confirmatory for second-stage disease; sensitivity is increased by centrifugation.
Antigen detection	Not relevant for *T. brucei rhodesiense* due to large numbers of trypomastigotes in blood	Diagnostic method much more relevant for *T. brucei gambiense* infections
Antibody detection	No equivalent to CATT available for screening for *T. brucei rhodesiense*; development and testing under way for agglutination card tests and particle agglutination tests; immunofluorescence tests and ELISA for antibody available, but the sensitivity varies	Immunofluorescence tests and ELISA available in reference centers (highly skilled individuals are required for testing and interpretation)
Molecular methods	Molecular markers (serum resistance-associated gene, *T. brucei rhodesiense*; receptor-like flagellar pocket glycoprotein, *T. brucei gambiense*) may be helpful in monitoring the distribution of both species.	Could also be helpful for targeting therapy for cattle infected with *T. brucei rhodesiense*, as well as distinguishing species in humans exposed to both organisms.

Suramin is the drug of choice for treating the hemolymphatic stage *of T. brucei rhodesiense* infections. When suramin is used, a test dose should be given to the patient to ensure that the drug can be tolerated. It should not be given to patients with renal disease, and if protein, RBCs, or casts are detected in the urine, treatment should be stopped. Suramin is given intravenously; its side effects include nausea, vomiting, loss of consciousness, seizures, pruritus, edema, and hepatitis. Although rare, fatalities have been reported during suramin therapy. Suramin can be obtained under an IND protocol from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, GA, 30333; 404-639-3670 (evenings, weekends, or holidays: 404-639-2888) (32).

Melarsoprol is capable of penetrating the blood-brain barrier and is the drug of choice when *T. brucei rhodesiense* CNS disease is suspected; it is effective for both hemolymphatic and neural stages. Melarsoprol has been used for CNS involvement for years; however, relapse occurs in up to 6% of patients. Arsenic-related encephalopathy occurs



Algorithm 28.1 African trypanosomiasis.
doi:9781555819002.ch28.Alg1

in 10% of patients; prednisolone treatment may help reduce the incidence. The mortality due to this side effect depends on whether supportive treatment is given, but it is often above 50% (33). Resistance of both *T. brucei gambiense* and *T. brucei rhodesiense* to melarsoprol has been noted. Melarsoprol can be obtained under an IND protocol from the CDC Drug Service (32). All treated patients should be followed up twice yearly for at least 2 to 3 years. Specific drug dosage information is provided in chapter 36.

Epidemiology, Epidemics, and Prevention

The incidence of *T. brucei rhodesiense* infections is considerably lower than that of *T. brucei gambiense* infections, and the extent of distribution is smaller. East African trypanosomiasis is usually found in woodland and savannah areas away from human habitation. The tsetse fly vectors of Rhodesian trypanosomiasis are game feeders that may incidentally transmit the disease from human to human or animal to human. Because the infection results in acute rather than chronic disease, asymptomatic carriers are not a source of transmission as they are in Gambian trypanosomiasis. The disease is an occupational hazard for persons working in game reserves and a threat to visitors to game parks. *T. brucei rhodesiense* has been isolated from a variety of game animals (bushbuck, hartebeest, and lion) and domestic animals (cattle and sheep), a group that may prove to be the more important group of reservoir animals.

The incidence of East African trypanosomiasis is characterized by short epidemics interspersed with long periods of low (often undetectable) endemicity (40). The reasons for such outbreak situations have been widely discussed and debated. Studies of the composition of trypanosome strains present during epidemics have been directed at answering the following questions. (i) Are human-infective trypanosomes (*T. brucei rhodesiense*) distinct from those infecting animals (*T. brucei brucei*)? (ii) Are new strains responsible for the outbreaks? (iii) Are animals an important reservoir for strains that cause human disease? (iv) Can the occurrence of new outbreaks be traced to the movement of new "virulent" strains into the area?

Distinguishing the Two Organisms. Field data using restriction fragment length polymorphism analysis of repetitive DNAs and isoenzymes confirm that the subspecific distinction of *T. brucei rhodesiense* and *T. brucei brucei* appears to be correct (40). Although isoenzyme analysis had incorrectly detected taxonomy identity caused by convergent evolution, DNA patterns in restriction fragment length polymorphism indicate that strains of *T. brucei rhodesiense* from Zambia and from Kenya and Uganda are very different and have independent origins.

Animal Reservoirs of Sleeping Sickness. In the past, it was assumed that wild animals, particularly bushbuck, were the most important reservoir host of the parasites. More recently, cattle have been implicated in several outbreaks (40). Data show that between 21 and 33% of infected domestic animals (particularly cattle) can be infected with trypanosomes that are also infective for humans during an epidemic. Probabilities of transmission indicate that a fly infected with *T. brucei rhodesiense* is five times more likely to have picked up that infection from domestic cattle than from a human.

"New Strains" and Epidemics. In looking at changes in the ecology of a region, the appearance of a new strain, and the generation of new zymodemes by genetic exchange, it appears that there is little evidence to support the idea of new strains as a mechanism for the generation of epidemics but that the spread of a single existing strain group is more likely to be responsible.

Movement of People and Trypanosomes. It was originally thought that *T. brucei rhodesiense* originated in Zambia in the early 1900s and spread north to cause epidemics in Uganda and Kenya in the 1940s. However, analysis of trypanosomes from various locales does not support this idea. It is more likely that the origins of Rhodesian sleeping sickness in the Uganda foci were there all the time and actually coexisted with the outbreaks in Zambia (40).

Model for the Origin and Maintenance of Disease Foci in East Africa. A model can be proposed as follows: (i) each sleeping sickness focus has a single strain of human-infective trypanosome associated with it; (ii) this strain is maintained within the ecosystem of the focus, even in times of endemicity; and (iii) when favorable epidemiologic conditions are present, this strain generates an epidemic (2). A number of stages can be involved: (i) a new genotype arises that can be maintained in the population; (ii) once a transmission cycle is established, selection may maintain a single human-infective strain or a small number of such strains; and (iii) epidemic selection of this strain may occur when the tsetse fly biting rate increases. When the overall density of the infected human population increases, a full-blown epidemic caused by a single trypanosome strain occurs. A period of endemicity then follows as control measures decrease the number of cases. The human-infective strains are left circulating until such time as ecologic changes trigger another epidemic.

Control Measures. Because Rhodesian trypanosomiasis is a zoonosis, control measures are more difficult to attain than with the Gambian form. In the past, reduction or elimination of game animals has been a major control method, although more recent information suggests that cattle are more relevant as reservoir hosts. Other measures used were the reduction of human contact in areas of endemic infection, reduction of vegetation around human settlements, insecticide spraying, use of fly traps, and prophylactic treatment of domestic animals. Tourists are usually not at great risk unless they are traveling and spending long periods in rural areas of eastern and central Africa. Persons visiting areas where the disease is endemic should avoid tsetse fly bites by wearing protective clothing (long-sleeved shirts and long pants); khaki or olive-colored clothing is optimal, since the tsetse fly is attracted to bright colors and very dark colors. Because heavy clothing is not always practical owing to heat and humidity, other measures, including the use of insect repellents, bed netting, and screens, are recommended. It has also been recommended not to ride in the back of jeeps, pickup trucks, or other open vehicles; the tsetse fly is attracted to the dust created by moving vehicles and wild animals. Bushes should be avoided, since the tsetse fly is less active during the hot period of the day and rests in bushes but will bite if disturbed.

With constant political change in Africa, vector control, mass treatment, prophylaxis, and population movement tracking will remain very difficult. Unfortunately, constant monitoring of areas of endemic infection is very difficult to accomplish and trained personnel continue to be scarce as other issues take priority.

American Trypanosomiasis

Trypanosoma cruzi

American trypanosomiasis (Chagas' disease) is a zoonosis caused by *T. cruzi*, which was discovered in the intestine of a triatomid bug in Brazil in 1909 by Carlos Chagas, who described the entire life cycle in reservoir hosts. After infected bugs were allowed to feed on a monkey, the trypomastigote form was found in the blood of the animal. Chagas then found the organisms in the blood of a child who had fever, anemia, and enlargement of the lymph nodes; he proved that the parasites were the cause of this common illness endemic in areas of Brazil. It is interesting that this represents the first example where an animal parasite causing the disease and the insect vector were discovered prior to the disease itself. Based on his assumption that the organism multiplies by schizogony, Chagas named the parasite *Schizotrypanum cruzi*, a name that is still used by some. *T. cruzi* causes an acute or chronic parasitemia and invades the cells of many organs (e.g., the heart, esophagus, and colon). Chagas' disease has been found in a 15th- or 16th-century Peruvian mummy with megacolon and megaesophagus. It is one of the major health problems in Latin American countries.

It is estimated that 100 million persons are at risk of infection; between 16 million and 18 million are actually infected. There are approximately 200,000 new cases each year and 50,000 deaths per year due to Chagas' disease. In certain areas of endemic infection, approximately 10% of all adult deaths are due to Chagas' disease. Two-thirds of reported cases occur in the Southern Cone countries (Argentina, Bolivia, Brazil, Chile, Paraguay, southern Peru and Uruguay), the stimulus for the development of the Southern Cone Initiative, one of the largest disease control programs ever established (41). The southern United States, particularly Texas, is also now identified as having a number of cases. The geographic distribution of *T. cruzi* infection overlaps that of *T. rangeli* infection; therefore, the trypomastigotes may be misidentified (Table 28.5).

Using a number of molecular markers, the *T. cruzi* population has been divided into a number of evolutionary lineages, also called discrete typing units. Some markers allow the distinction of two or three major lineages, while other experimental strategies, such as random amplified polymorphic DNA and multilocus isoenzyme electrophoresis, support the distinction of six subdivisions originally designated as DTUs I, IIa, IIb, IIc, IId, and IIe. Recently, this nomenclature was revised as follows: TcI, TcII (former TcIIb), TcIII (IIc), TcIV (TcIIa), TcV (TcIId), and TcVI (TcIIe) (42). Prior to 2005, epidemiological and immunological data suggested that chronic infections occurring in Brazil and Argentina were caused primarily by *T. cruzi* II strains. Using PCR techniques, this hypothesis has been confirmed, and future studies can establish which strains are responsible for Chagas' disease in other geographic locations (43, 44). Infections by the same species cause different clinical outcomes, which is believed to be determined, at least in part, by the genetic background of the parasite (infection by different strains).

TABLE 28.5 Characteristics of American trypanosomiais

Characteristic	*Trypanosoma cruzi*	*Trypanosoma rangeli*
Vector	Reduviid bug	Reduviid bug
Primary reservoirs	Opossums, dogs, cats, wild rodents	Wild rodents
Illness	Symptomatic (acute, chronic)	Asymptomatic
Diagnostic stage		
Blood	Trypomastigote	Trypomastigote
Tissue	Amastigote	None
Recommended specimens	Blood, lymph node aspirate, chagoma	Blood, but organisms rarely recovered

Life Cycle and Morphology

The disease is transmitted to humans through the bite wound caused by reduviid bugs (triatomids, kissing bugs, or conenose bugs) (Fig. 28.11 and 28.12). Humans are infected when metacyclic trypomastigotes are released with the feces while the insect is taking a blood meal and the feces are rubbed or scratched into the bite wound or onto mucosal surfaces such as eyes or mouth, an action stimulated by the allergic reaction to the insect's saliva. The organisms can also be transmitted as congenital infections, by blood transfusion, or by organ transplantation. On entry into the body, the metacyclic forms invade local tissues, transform to the amastigote stage, and begin to multiply within the cells. The local inflammatory process continues, forming the primary lesion, the chagoma, which blocks the lymphatic capillaries and causes edema (Fig. 28.13).

A number of receptors have been identified that are involved in the invasion process of the trypomastigote into the host cell. In humans, *T. cruzi* can be found in two forms, amastigotes and trypomastigotes (Fig. 28.3, 28.14, and 28.15). The trypomastigote does not divide in the blood but carries the infection to all parts of the body. The amastigote form multiplies within virtually any cell, preferring cells of mesenchymal origin such as reticuloendothelial, myocardial, adipose, and neuroglial cells. The parasites also occur in histiocytes of cutaneous tissue and in cells of the epidermis, as well as in the intestinal mucous membrane.

The trypomastigote (Fig. 28.14) is spindle shaped and approximately 20 µm long, and it characteristically assumes a C or U shape in stained blood films. Trypomastigotes occur in the blood in two forms, a long slender form and a short stubby one. The nucleus is situated in the center of the body, with a large oval kinetoplast located at the posterior extremity. The kinetoplast consists of a small blepharoplast and a large oval parabasal body. A flagellum arises from the blepharoplast and extends along the outer edge of an undulating membrane until it reaches the anterior end of the body, where it projects as a free flagellum. When the trypomastigotes are stained with Giemsa stain, the cytoplasm stains blue and the nucleus, kinetoplast, and flagellum stain red or violet.

On invasion of a cell, the trypomastigote loses its flagellum and undulating membrane and divides by binary fission to form an amastigote (Fig. 28.15). The amastigote continues to divide and eventually fills and destroys the

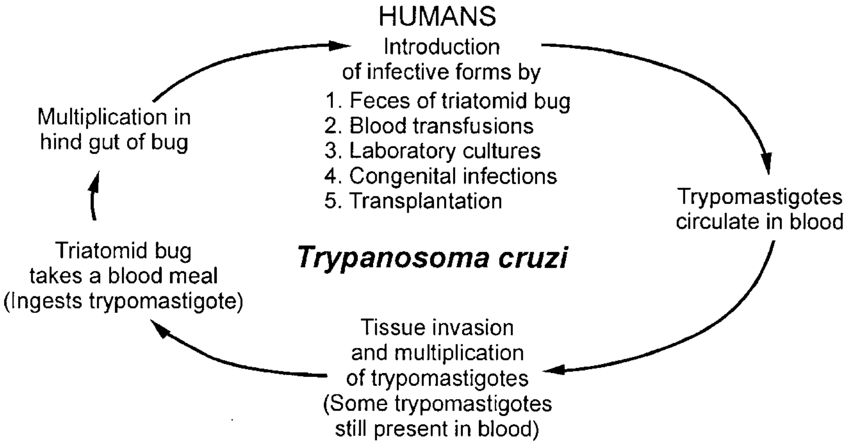

HUMANS
Introduction
of infective forms by
1. Feces of triatomid bug
2. Blood transfusions
3. Laboratory cultures
4. Congenital infections
5. Transplantation
Trypomastigotes
circulate in blood
Tissue invasion
and multiplication
of trypomastigotes
(Some trypomastigotes
still present in blood)
Triatomid bug
takes a blood meal
(Ingests trypomastigote)
Multiplication in
hind gut of bug
Trypanosoma cruzi

Figure 28.11 Life cycle of *Trypanosoma cruzi*. doi:10.1128/9781555819002.ch28.f11

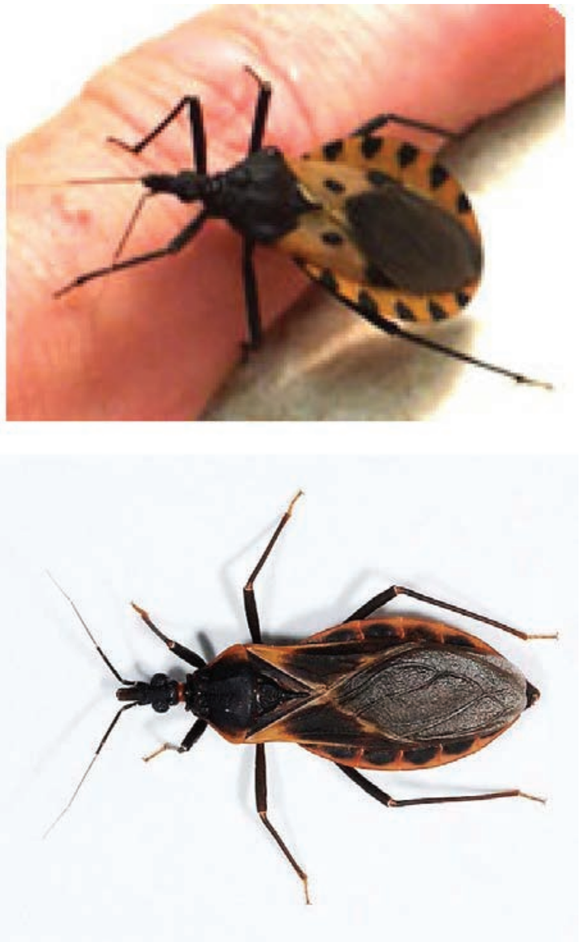


Figure 28.12 Triatomid/reduviid/"kissing"/assassin bugs (courtesy of the CDC Public Health Image Library; lower from J. G. Hindebrand). doi:10.1128/9781555819002.ch28.f12

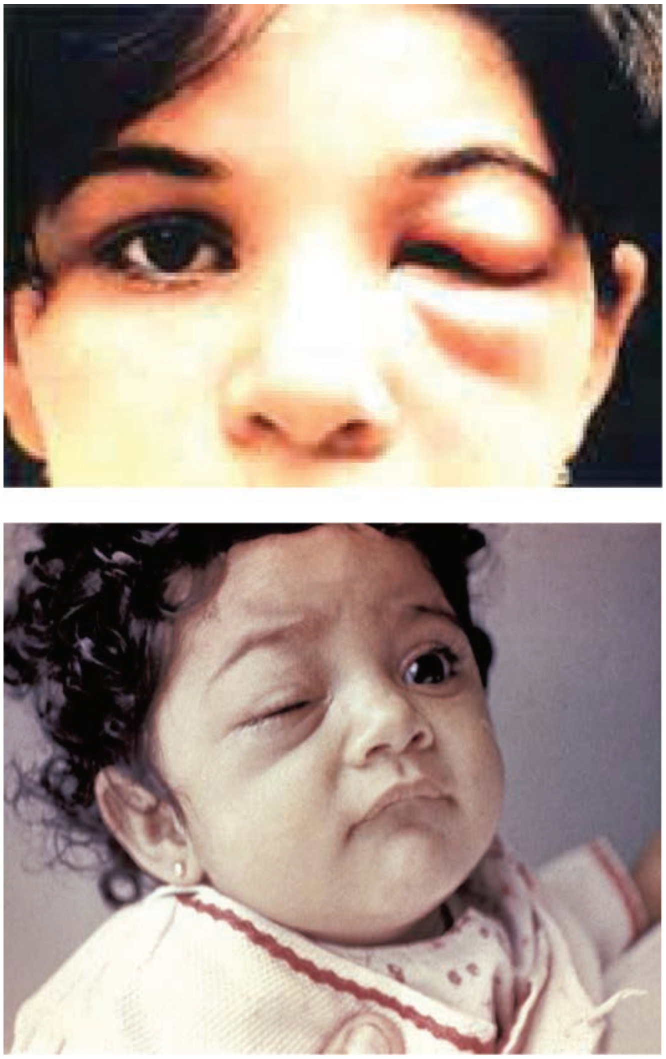


Figure 28.13 Romaña's sign. The assumption is that this is the bite site of the vector, the triatomid bug; unilateral conjunctivitis, palpebral and periorbital edema. (Upper, courtesy of the CDC Public Health Image Library; lower, courtesy of the Armed Forces Institute of Pathology.) doi:10.1128/9781555819002.ch28.f13

infected cell. Both amastigote and trypomastigote forms are released from the cell. The amastigote is indistinguishable from those found in leishmanial infections. It is 2 to 6 µm in diameter and contains a large nucleus and rod-shaped kinetoplast that stains red or violet with Giemsa stain. The cytoplasm stains blue. Only the trypomastigotes are found free in the peripheral blood.

Trypomastigotes are ingested by the reduviid bug as it obtains a blood meal. The trypomastigote transforms into an epimastigote which multiplies in the posterior portion of the midgut (Fig. 28.16). After 8 to 10 days, metacyclic trypomastigotes develop from the epimastigotes and are passed in the feces to infect humans when rubbed into the insect's puncture wound or rubbed onto exposed mucous membranes.

Clinical Disease

In addition to contracting *T. cruzi* infections through the insect's bite wound or exposed mucous membranes, persons can be infected by blood transfusion, organ transplantation, placental transfer, and accidental ingestion of parasitized reduviid bugs. The clinical syndromes associated with Chagas' disease can be broken down into acute, indeterminate, and chronic stages. The acute stage is the result of the first encounter of the patient with the parasite, whereas the chronic phase is the result of late sequelae. In children younger than 5 years, the disease is seen in its severest form, whereas in older children and adults, the disease is milder and is commonly diagnosed in the subacute or chronic form rather than in the acute form. Overall, the incubation period in humans is about 7 to 14 days but is somewhat longer in some patients.

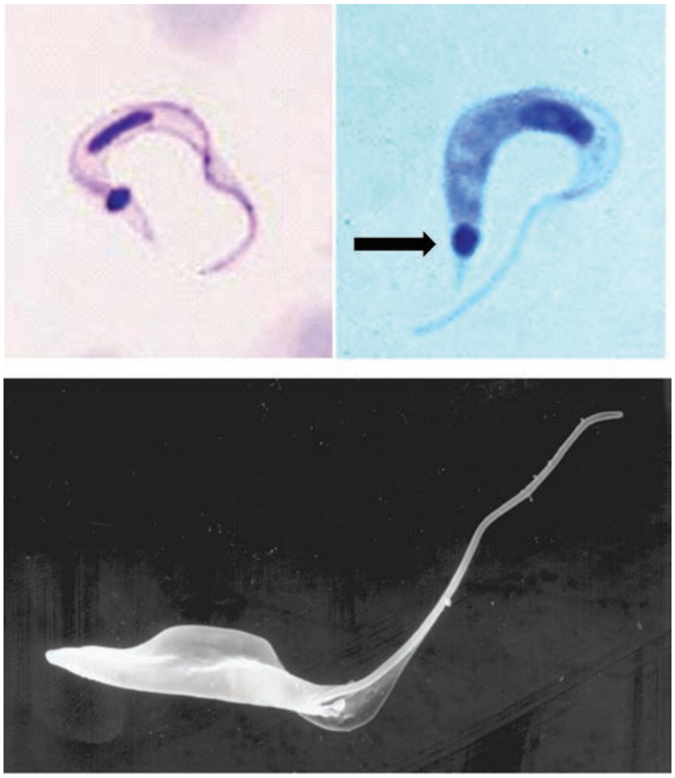


Figure 28.14 (Top, left and right) *Trypanosoma cruzi* trypomastigotes in a peripheral blood smear. Note the large kinetoplast (arrow). (Bottom) Scanning electron micrograph of a trypomastigote of *T. cruzi* (courtesy of David T. John).
doi:10.1128/9781555819002.ch28.f14

Early Disease. A localized inflammatory reaction of variable intensity may ensue at the infection site. In most cases, the reaction is mild and may not be apparent. An erythematous subcutaneous nodule (chagoma), seen most frequently on the face, may form. The chagoma is painful and may take 2 to 3 months to subside. In early stages of infection, amastigotes or trypomastigotes may be aspirated from the chagoma; if the route of inoculation is the ocular mucosa, edema of the eyelids and conjunctivitis may occur (Romaña's sign) (Fig. 28.13). The edema does not pit on pressure, and the skin is dry. Although Romaña's sign is thought to be almost pathognomonic for early Chagas' disease, in an analysis of 300 patients with early disease, unilateral periorbital edema was found in only 48.7% of patients (38). The infective stages spread to the regional lymph nodes, which become enlarged, hard, and tender. Similar chagoma lesions may develop in other body areas as the infection spreads hematogenously. Trypomastigotes appear in the blood about 10 days after infection and persist through the acute phase. These stages are rare or absent during the chronic phase.

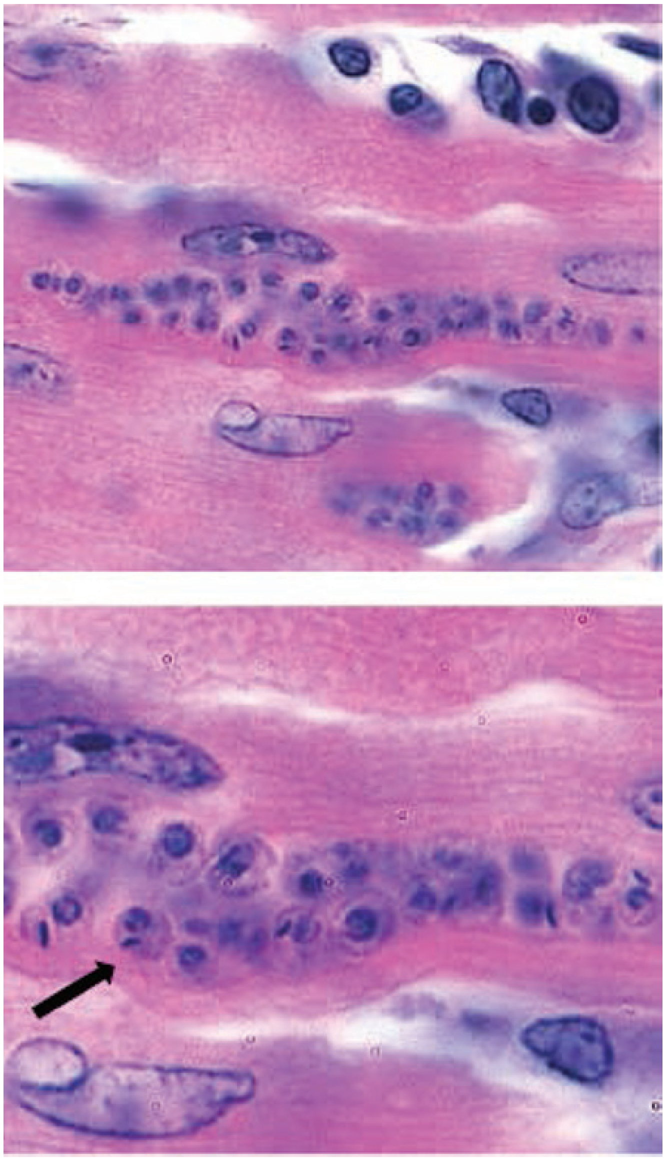


Figure 28.15 *Trypanosoma cruzi* amastigotes in cardiac muscle; note the actual amastigote (nucleus, kinetoplast) (arrow). Lower panel is higher magnification, hematoxylin and eosin stain. (Courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch28.f15

Acute-Stage Disease. Acute-stage symptoms only occur in about 1% of patients, are usually seen in younger children, and are less obvious in older individuals because of the nonspecific nature of the symptoms and the lack of availability of health care. In general, symptoms last for 4 to 8 weeks and then may subside, even without therapy. Acute systemic signs occur around week 2 to 3 of infection and are characterized by high fevers, which may be intermittent, remitting, or continuous; hepatosplenomegaly; myalgia; erythematous rash; acute myocarditis; lymphadenopathy; keratitis; and subcutaneous edema of the face, legs,

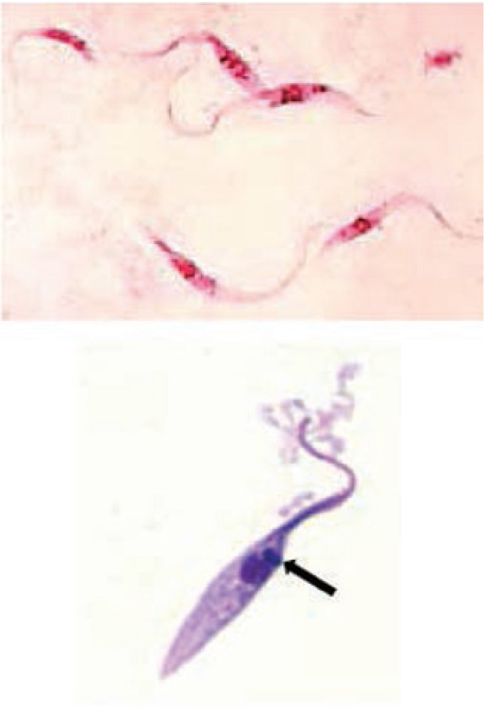


Figure 28.16 Epimastigotes of *Trypanosoma cruzi* from culture. Note that the kinetoplast is anterior to the nucleus (arrow). (Lower image courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch28.f16

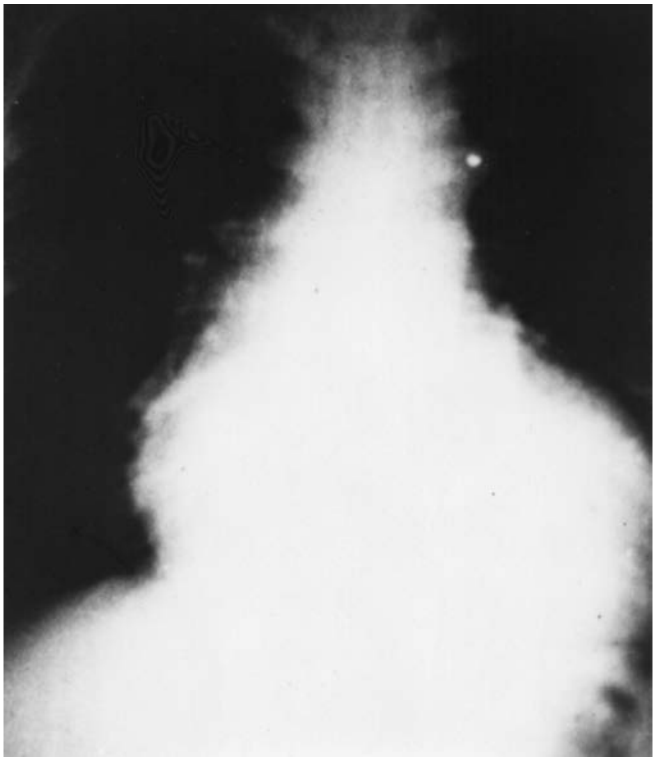


Figure 28.17 An enlarged heart in a 46-year-old man 13 days after onset of acute Chagas' disease. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch28.f17

and feet. There may be signs of CNS involvement including meningoencephalitis, which has a very poor prognosis. Myocarditis is manifested by electrocardiographic changes, tachycardia, chest pain, and weakness. Amastigotes proliferate within and destroy the cardiac muscle cells, leading to conduction defects and a loss of heart contractility (Fig. 28.17 to 28.19). Death may occur due to myocardial insufficiency or cardiac arrest. In infants and very young children with acute Chagas' disease, swelling of the brain can develop, causing death. Also, in some areas of endemic infection, a high frequency of early electrocardiographic abnormalities in children who are *T. cruzi* seropositive suggests a rapid evolution from infection to disease. Under such conditions, a public health chemotherapy program targeted to this population would be recommended (45).

All patients with acute Chagas' disease probably show some degree of myocarditis, which is apparently directly related to the presence of parasitic antigens. Both CD4 and CD8 cells participate in the pathogenesis of myocarditis, possibly leading to chronic heart failure (46). The numbers of CD4 and CD8 cells may actually correlate better with myocarditis than does the presence of parasite antigens.

Indeterminate Stage. Patients in the acute stage of Chagas' disease may die within a few weeks or months, may recover, or may enter the chronic stage of infection. The patient may have a subpatent parasitemia and will develop antibodies to a variety of *T. cruzi* antigens. Chronic Chagas' disease is diagnosed more commonly than the acute disease. The chronic stage may be initially asymptomatic (indeterminate stage), and even though trypomastigotes are seldom seen in peripheral blood, transmission by blood transfusion is a serious problem in areas where the disease is endemic. There are regional variations in disease severity, which may account for the geographic differences in morbidity. Approximately 8 to 10 weeks after infection, the indeterminate stage begins, during which the patients do not have any symptoms. Patients infected with low-virulence strains may remain infected for years with few to no problems.

Chronic Stage. Chronic Chagas' disease demonstrates close interaction between the parasite and the host, causing a number of different clinical syndromes. Approximately 30% of patients develop chronic Chagas' disease and experience cardiomyopathy, megacolon, and megaesophagus. Symptoms of the chronic stage are related to the damage

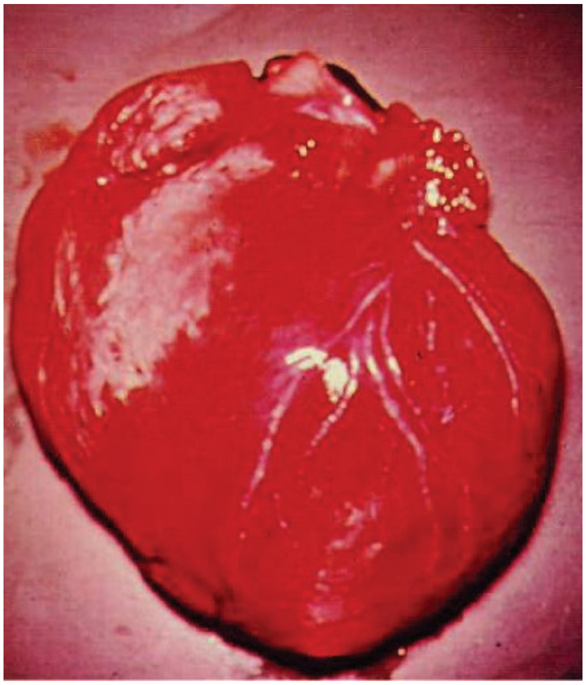


Figure 28.18 Acute chagasic myocarditis. (Original photograph by B. H. Kean; from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch28.f18

sustained during the acute stage of the disease, the state of the host's immune system, and the inflammatory response. Other contributing factors include the host genetic background, environmental and social factors, the genetic composition of the parasite, mixed infections, and reinfections (47). The inflammatory response undergoes periods of exacerbation and is probably responsible for neuronal damage, microcirculatory changes, heart matrix deformations, and possible cardiac failure (48). Selenium deficiency has also been linked to some cardiomyopathies and appears to be a biological marker for Chagas' disease and related to progressive pathology (49).

Chronic Chagas' disease may develop years or decades after undetected infection or after the diagnosis of acute disease. The most frequent clinical sign of chronic Chagas' disease in 25 to 30% of patients is cardiomyopathy manifested by cardiomegaly and conduction changes. This may present as congestive heart failure, thromboembolic phenomena, cardiac arrhythmias and sudden death. Cardiac pathology includes mononuclear inflammatory infiltrate, focal myocarditis, epicarditis, and neuroganglionitis, associated with variable focal fibrosis and widely variable autonomic dysfunction. Apparently, the immune inflammatory response causes the autonomic dysfunction, which may lead to life-threatening arrhythmias and sudden death. A review of the potential influence of associated risk factors

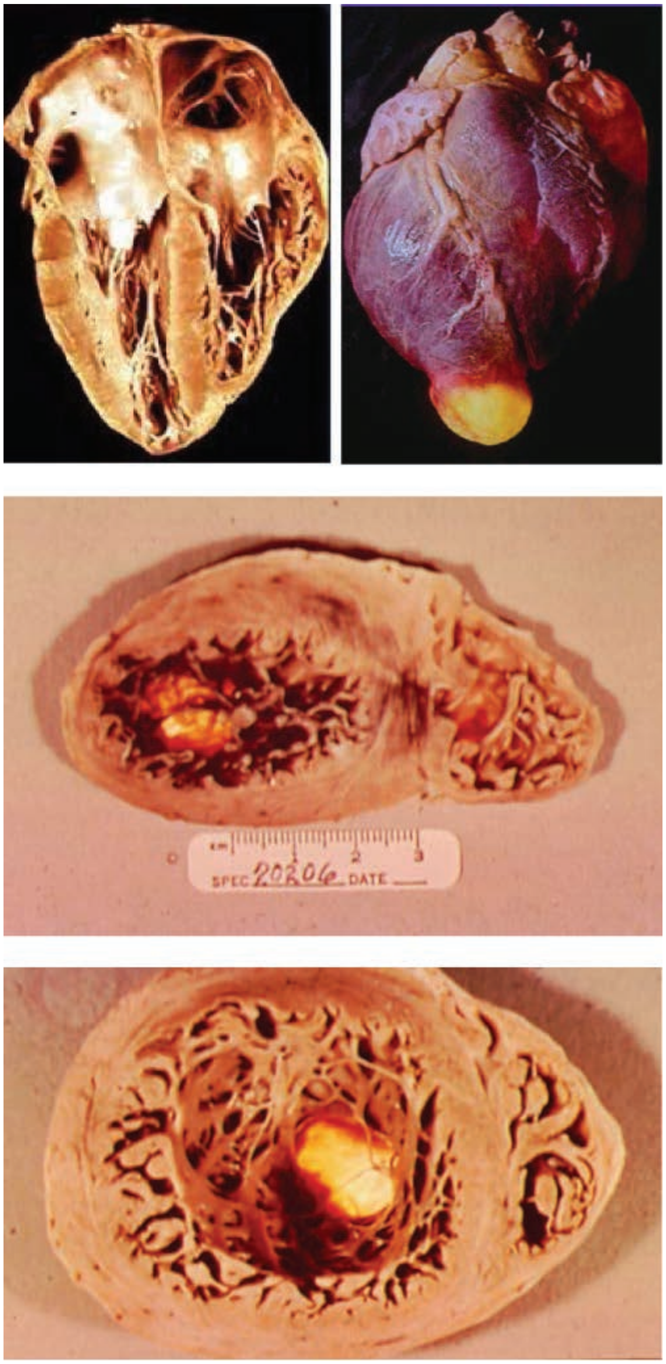


Figure 28.19 (Top left) Open chagasic heart with thinning of wall; (right) note the dilation of the apical myocardium. (Middle) Aneurysmal dilatation and thinning of the apical myocardium, plus marked concentric muscular hypertrophy. (Bottom) Same heart with thin apical myocardium. Note the light shining through the thinned muscle. (Upper right, middle, and bottom photographs by J. H. Edgecome, from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch28.f19

such as smoking, alcoholism, obesity, and hypertension on the establishment of chronic chagasic cardiomyopathy showed that these factors apparently were not associated with increased risk (50).

It is not understood why some patients in the indeterminate phase of *T. cruzi* infection develop chronic disease, while most remain asymptomatic. Although genetic susceptibility of the host is probably a factor, it has been also speculated that the phylogenetic lineage of the infecting *T. cruzi* strain and/or heterogeneity of the parasite population may also influence the varying clinical manifestations of Chagas' disease. It appears that the *in vivo* persistence of *T. cruzi* over many decades is related to parasite invasion of a large number of different cell types. The intracellular location of *T. cruzi* in vertebrates and the remarkable plasticity of its infective developmental forms—alternating between highly motile trypomastigotes and intracellularly replicating amastigotes—are likely to play a central role in ensuring parasite survival, despite the presence of immunity (51).

Autoimmunity Hypothesis (Pros and Cons). The merits of the autoimmunity hypothesis for the pathogenesis of Chagas' disease have been debated for years. Although several auto- or cross-reactive antibodies have been identified in chagasic patients, no convincing evidence exists for their ability to induce the lesions seen in infected patients (52). Because so few organisms are isolated or seen in heart tissue, much of the cardiac tissue destruction is thought to be related to autoimmune antibodies, possibly due to cross-reactivity with related *T. cruzi* antigens (molecular mimicry) (53–60). There is massive lymphocyte polyclonal activation, which results in the generation of autoantibodies cross-reacting with parasites and host tissues. Degenerative abnormalities occur in cardiac, esophageal, and colon tissues, leading to megasyndrome. The mechanism that triggers the megasyndrome is unknown. An antibody that reacts with endocardium, vascular structures, and interstitium of striated muscle (EVI antibody) has been detected in patients with Chagas' disease. The EVI antibody has been detected in a significant number of patients with chagasic cardiomyopathy. Antibodies that react with the Schwann sheaths of somatic and autonomic peripheral nerves have also been detected. A correlation of prevalence and serologic titers was detected for the antinerve and EVI antibodies. These antibodies could be absorbed from the sera by using *T. cruzi* epimastigotes, which suggests the presence of cross-reacting antibodies between parasite and hosts. These antibodies may play a large role in the denervation that occurs with megaesophagus, megacolon, and cardiomegaly.

Abnormalities of the coronary microcirculation have been seen in patients with acute chagasic myocarditis and may cause transient ischemia consistent with the hypothesis of a microvascular factor as a cause of myocardial changes. These changes are linked to endothelium structure and integrity. If the protective capability of the endothelial cells is reduced, vasospasm and platelet aggregation within coronary microvessels may lead to focal pathology.

Various mechanisms have been proposed for the pathogenesis of chronic chagasic cardioneuromyopathy. Chronic interaction of chagasic IgG with myocardial muscarinic acetylcholine receptors, behaving as a muscarinic agonist, might lead to cell dysfunction or tissue damage. Also, these antibodies could produce desensitization, internalization, or degradation of cardiac muscarinic acetylcholine receptors, explaining the progressive blockade of these receptors in the myocardium with parasympathetic denervation. Data also suggest that cardiac autonomic impairment and early myocardial damage involving the right ventricle are independent phenomena in Chagas' disease (61). Cross-reactive antibodies to cardiac and parasite immunogenic glycolipids have been detected and may be involved in Chagas' disease autoimmunity (62). Increases in the levels of asialoganglioside and monosialoganglioside-reactive antibodies have also been detected in patients with chronic Chagas' disease (63). Molecular mimicry has also been proposed on the basis of data related to cross-reactive antibodies between cardiac myosin and the *T. cruzi* B13 protein.

Human chronic Chagas' cardiomyopathy appears to fulfill many of the criteria for autoimmune diseases, similar to insulin-dependent diabetes mellitus or rheumatoid arthritis. However, validation of the target antigens must involve the induction of cardiac lesions after immunization or passive transfer of antigen-specific T cells. Aberrant T-cell activation may also play a role in cardiac injury (64). Not all researchers agree that autoantibodies play a role in pathogenesis (65, 66). Also, some propose a multifactorial or "combined" theory to explain the sequence of events leading to chronic myocarditis. The actual clinical course may vary from heart failure to a slow but continuing loss of cardiac function, with possible ventricular rupture and thromboemboli.

Tissue Involvement Other than Cardiomyopathy. Although it is less common than cardiac involvement, patients from certain areas are more likely to have dilation of the digestive tract with or without cardiomyopathy. These symptoms are most frequently seen in the esophagus and colon as a result of neuronal destruction (Fig. 28.20 and 28.21). Megaesophagus, characterized by dysphagia, chest pain, regurgitation, and malnutrition, is related to loss of contractility of the lower esophagus. Hypersalivation may occur, leading to aspiration with repeated bouts of aspiration pneumonia. Leiomyosarcoma of the esopha-

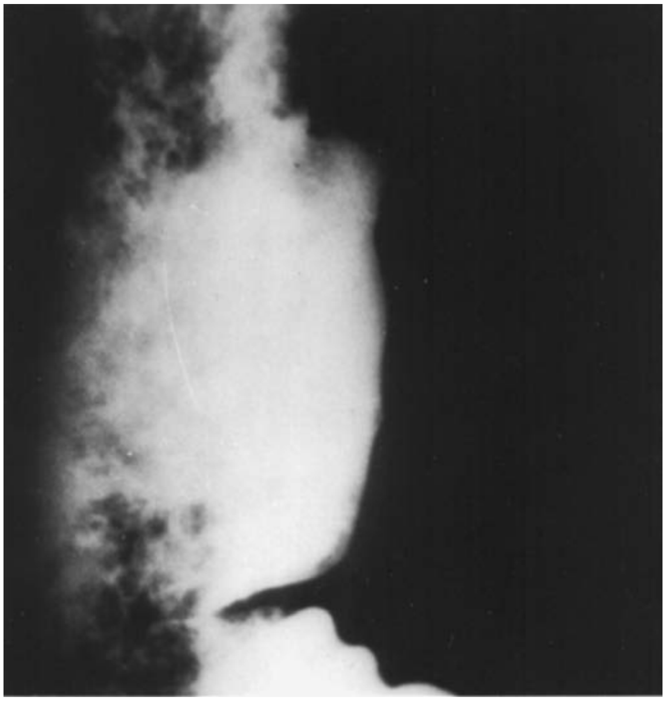


Figure 28.20 Chagasic megaesophagus. (Original photography by D. N. Reifsnyder, from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch28.f20

gus in a patient with chagasic megaesophagus has been reported (67). Megacolon results in constipation, abdominal pain, and the inability to discharge feces. In some individuals, there may be acute obstruction leading to perforation, septicemia, and death. Cases of colon cancer have also been associated with chagasic megacolon.

Congenital Transmission. Congenital transmission can occur in both the acute and chronic stages of the disease. Common clinical findings of congenital infection are stillbirth, low birth weight, myocarditis, neurologic alterations, and death shortly after birth. Infants of seropositive mothers should be monitored for up to 1 year after birth. Monitoring should include examination of blood for parasites and serologic tests.

Immunocompromised Patients. Chagas' disease has been found in HIV-positive patients, and neurologic sequelae due to *T. cruzi* have been major findings in these patients (68). Individuals who were previously infected with *T. cruzi* and later become positive for HIV are at risk of reactivation of their Chagas' disease. These patients develop a severe multifocal or diffuse meningoencephalitis with numerous tissue parasites (69). Meningoencephalitis is a rare event in non-AIDS patients and is seen most frequently in immunocompetent children younger than 4

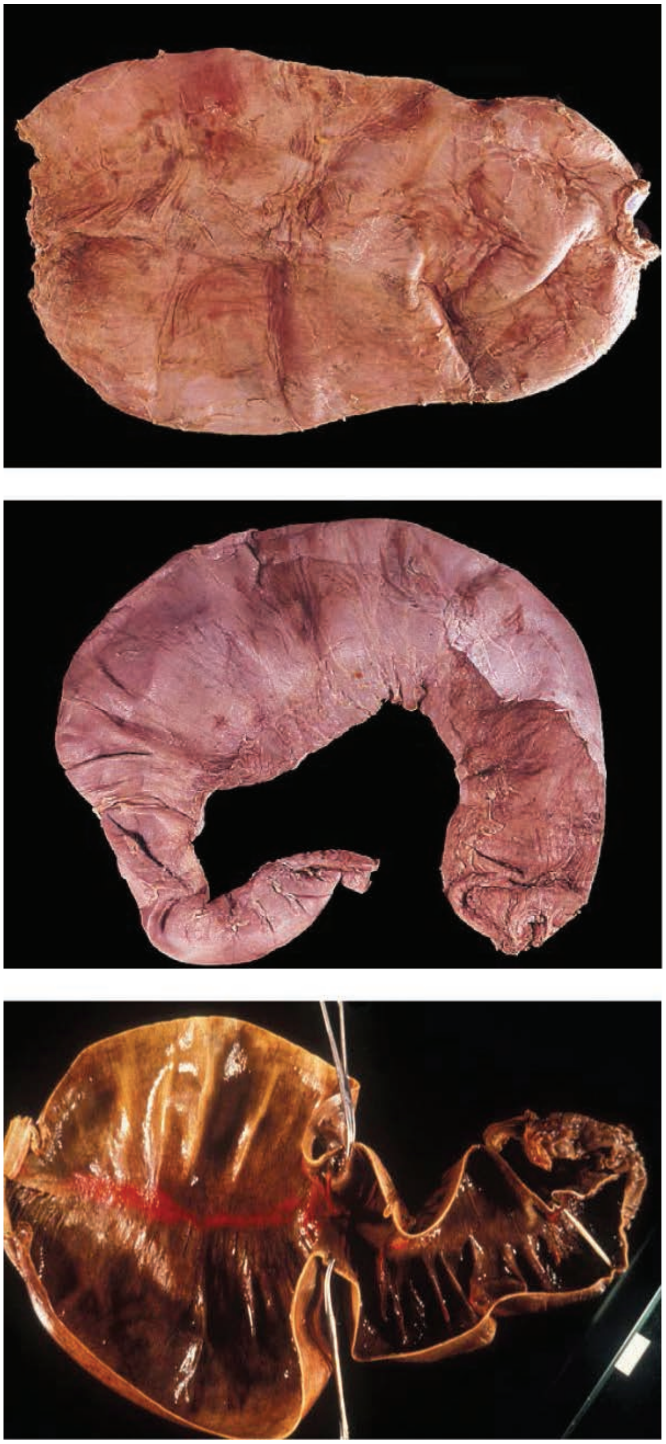


Figure 28.21 (Top) Megaesophagus in 34-year-old Brazilian patient with chronic Chagas' disease; (middle) megacolon in an adult male with Chagas' disease (Armed Forces Institute of Pathology photographs). (Bottom) Megacolon, cut image (original photograph by B. H. Kean; from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). doi:10.1128/9781555819002.ch28.f21

years. Because of the immunodeficiency associated with HIV infections, concomitant infection with *T. cruzi* may be difficult to recognize, particularly in individuals who have moved to areas where the disease is not endemic. *T. cruzi* infection may also have a protracted asymptomatic course in immunosuppressed HIV patients (70). Although a positive blood smear has been considered the key indicator of Chagas' disease reactivation in immunocompromised patients with chronic disease, this finding may occur late, rather than early, in the reactivation process (71). Apparently, there is no association between given *T. cruzi* genotypes and specific clinical forms of Chagas' disease-HIV associations (72).

Recrudescence of *T. cruzi* infections in immunosuppressed patients, particularly transplantation patients, is a grave concern. Transplant recipients can also become infected through receipt of infected organs (73). For patients with end-stage Chagas' cardiomyopathy, heart transplantation is an option which has had variable success (74). Reactivation of the disease with the development of cutaneous lesions has been seen. Bone marrow transplant recipients are also at risk of Chagas' disease due to reactivation or transfusion. Prophylactic treatment of these patients has led to favorable outcomes.

In studies of the Indians in western Paraguay, there appears to be a link between *T. cruzi* seropositivity and human T-cell leukemia virus type 2 (HTLV-2) infection. In this particular group, an individual infected with HTLV-2 is 2.28 times more likely to be *T. cruzi* positive than an HTLV-2-negative person. The public health significance of these findings is emphasized by the fact that approximately 18 million people are infected with *T. cruzi* in the Americas and the frequency of HTLV-2 infection continues to increase (75).

The high levels of IgM present in patients with African trypanosomiasis are not found in patients with either acute or chronic Chagas' disease. Antigenic variation, which is characteristic of infections with African trypanosomes, is less common in *T. cruzi* disease. In spite of humoral and cellular immunity against *T. cruzi*, the infection is able to persist in the host. As occurs in African trypanosomiasis, there is suppression of the immune response, with decreases in the levels of gamma interferon and interleukin-2, which help regulate cellular and humoral responses.

Diagnosis

Health care personnel working with specimens from patients suspected of having Chagas' disease must use standard precautions. Trypomastigotes are highly infectious, and certain strains of the parasite are more virulent than others. The diagnosis of Chagas' disease should be considered if there is a history of consistent exposure to reduviid bug bites, residence in or travel to areas where the disease is endemic, laboratory accident, or recent blood transfusion in an area where the disease is endemic. Chagomas appear similar to injury caused by trauma, insect bites, or bacterial and mycotic infections. The differential diagnosis of acute Chagas' disease should include brucellosis, endocarditis, salmonellosis, schistosomiasis, toxoplasmosis, tuberculosis, connective tissue diseases, and leukemia. Chronic Chagas' disease with cardiomyopathy may be confused with endocarditis, ischemic heart disease, and changes associated with rheumatic heart disease.

Routine Laboratory Methods. Acute Chagas' disease should be suspected in any individual from an area where the disease is endemic who develops an acute febrile illness with lymphadenopathy and myocarditis. The presence of a chagoma or Romaña's sign may be diagnostic; however, allergic reactions to insect bites may produce similar lesions. The definitive diagnosis depends on demonstration of trypomastigotes in the blood, amastigote stages in tissues, or positive serologic reactions (Table 28.5). Methods used for examination of blood are similar to those used for the diagnosis of African trypanosomiasis discussed earlier in this chapter. Trypomastigote stages (Fig. 28.14) may be easily detected in the blood in young children; however, in chronic disease, this stage is rare or absent except during febrile exacerbations. Trypomastigotes may be detected in blood by using thin and thick blood films or by buffy coat concentration techniques. The most sensitive methods for detection of trypomastigotes are concentration techniques such as the Strout method of buffy coat preparations (see chapter 7 for a discussion of concentration methods). With the Strout method, blood is collected without anticoagulant and allowed to clot. The serum is centrifuged at low speed to remove the remaining blood cells and then at a higher speed (600 × *g*) to concentrate the parasites in the sediment. Giemsa stain is often used for both trypomastigote and amastigote stages; however, any of the other blood stains are also acceptable.

Because of the overlap of geographic distribution, infections with *T. rangeli* trypomastigotes may have to be differentiated from those with *T. cruzi* trypomastigotes. *T. cruzi* trypomastigotes are usually C or U shaped on fixed blood films and have a large oval kinetoplast at the posterior end. *T. rangeli* trypomastigotes have a smaller kinetoplast a short distance from the posterior end, and there is no amastigote stage. In areas where kala azar occurs, amastigote stages (Fig. 28.15) appear similar, and infections with *Leishmania donovani* and *T. cruzi* must be differentiated. This can be done by PCR, immunoblotting, culture (epimastigote in *T. cruzi* versus promastigote in *L. donovani*), serologic tests, animal inoculation, or xenodiagnosis.

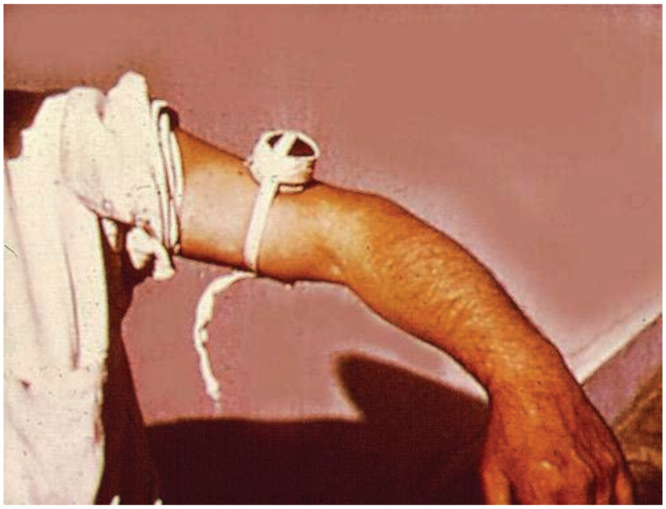
Aspirates from chagomas and enlarged lymph nodes can be examined for amastigotes and trypomastigotes. Histologic examination of biopsy specimens may also be done. Aspirates, blood, and tissues can also be cultured, which is valuable in detecting low-grade parasitemias. The medium of choice is Novy-MacNeal-Nicolle medium. Cultures should be incubated at 25°C and observed for epimastigote stages for up to 30 days before they are considered negative. If available, laboratory animals (rats or mice) can be inoculated and the blood can be observed for trypomastigotes.

In the chronic stage of Chagas' disease, trypomastigotes are very rare or absent in the peripheral blood except during febrile exacerbations. Diagnosis depends primarily on culture, xenodiagnosis, or serologic tests. Some individuals with chronic Chagas' disease may have a depressed humoral immune response, being serologically negative. This response has been correlated with specific zymodemes. Chronic disease should be considered in individuals from areas of endemic infection who show signs of cardiomegaly, cardiac conduction defects, severe constipation, or dysphagia.

Follow-up blood specimens should be reexamined 1 to 2 months after therapy. If available, xenodiagnosis may be performed (Fig. 28.22). The patient may also be monitored through serologic tests and electrocardiograms (see Algorithm 28.2).

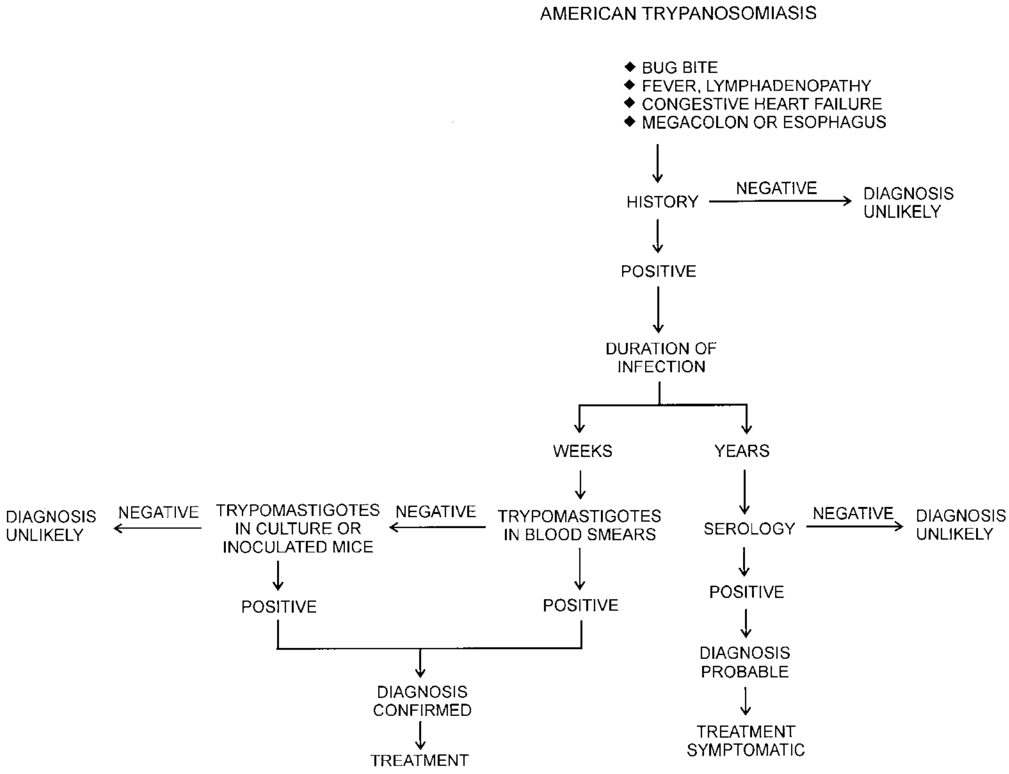
PCR. Although not routinely available except in specialized centers, PCR has been used to detect positive patients with as few as one trypomastigote in 20 ml of blood (76–78). PCR may be very useful for the diagnosis of patients with chronic Chagas' disease because of the lack of sensitivity and specificity of serologic tests and the lack of sensitivity of xenodiagnosis. PCR testing may be very useful for monitoring patients who have received therapy in order to validate cures (79, 80). It has also been used to diagnose congenital infections by detecting *T. cruzi* in neonatal blood (81). PCR has been used in combination with xenodiagnosis as a more sensitive replacement for the microscopic examination of triatomine fecal samples (82). Systematic monitoring of transplant recipients with organs from *T. cruzi*-infected donors has been very effective in providing an early diagnosis allowing timely therapy (83).

Figure 28.22 Xenodiagnosis for detection of positive Chagas' disease patient (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). doi:10.1128/9781555819002.ch28.f22



Immunoassays. Immunoassays have been used to detect antigens in the urine and sera of patients with congenital infections and those with chronic Chagas' disease (84). Determination of antigenuria can be valuable for early diagnosis of Chagas' disease and also for diagnosis of chronic cases in patients with conflicting serologic test results. A highly sensitive and specific chemiluminescent ELISA has been developed for blood bank screening and to monitor patients who are undergoing chemotherapy (85). Another ELISA using a recombinant antigen consisting of four different peptides has also been developed for screening blood donors and for epidemiologic studies and diagnosis (86).

Xenodiagnosis. In areas of endemic infection where reduviid bugs are readily available, xenodiagnosis can be used to detect light infections; this technique is most valuable for chronic infections when there are few trypomastigotes in the blood. Trypanosome-free bugs are allowed to feed on individuals suspected of having Chagas' disease. If organisms are present in the blood meal, the parasites multiply and can be detected in the bug's intestinal contents, which should be examined monthly for flagellated forms over a period of 3 months. To optimize this method, 20 third- or fourth-instar nymphs per day are allowed to feed on a patient for three consecutive days. A significant disadvantage of this technique is that not all triatomids are equally susceptible to infection and development of the epimastigote stage. Individuals who have been previously bitten by triatomids may be sensitized to their salivary secretions and may develop an anaphylactic reaction. Because the epimastigotes of *T. cruzi* and *T. rangeli* have heterogeneous morphologies, it may not be possible to make a definitive diagnosis microscopically. Alternative diagnostic techniques such as PCR and use of a monoclonal antibody may be needed, particularly in areas where there is geographic overlap (82).


AMERICAN TRYPANOSOMIASIS
◆ BUG BITE
◆ FEVER, LYMPHADENOPATHY
◆ CONGESTIVE HEART FAILURE
◆ MEGACOLON OR ESOPHAGUS
HISTORY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
DURATION OF INFECTION
WEEKS
YEARS
DIAGNOSIS UNLIKELY
NEGATIVE
TRYPOMASTIGOTES IN CULTURE OR INOCULATED MICE
NEGATIVE
TRYPOMASTIGOTES IN BLOOD SMEARS
SEROLOGY
NEGATIVE
DIAGNOSIS UNLIKELY
POSITIVE
POSITIVE
POSITIVE
DIAGNOSIS PROBABLE
DIAGNOSIS CONFIRMED
TREATMENT SYMPTOMATIC
TREATMENT

Algorithm 28.2 American trypanosomiasis. doi:9781555819002.ch28.Alg2

Serologic Testing. Serologic tests used for the diagnosis of Chagas' disease include complement fixation (Guerreiro-Machado test), indirect fluorescent-antibody tests, indirect hemagglutination tests, and ELISA (87). The use of synthetic peptides and recombinant antigens has improved the sensitivity and specificity of these diagnostic techniques, particularly in the diagnosis of congenital disease by using IgM and IgA antibody detection and in the evaluation of cure (88). Depending on the antigens used, cross-reactions have been noted to occur in patients with *T. rangeli* infection, leishmaniasis, toxoplasmosis, schistosomiasis, syphilis, leprosy, hepatitis, infectious mononucleosis, systemic lupus erythematosus, and rheumatoid arthritis. The Western blot method has been recommended for confirmatory serologic diagnosis of Chagas' disease (89). Another potential confirmatory assay using preserved protein antigens of *T. cruzi* discriminates chagasic from nonchagasic infections and may be useful for elucidating inconclusive results obtained by standard serologic tests; this is particularly relevant to blood bank testing (90). Long-lasting antibodies detected by a *trans*-sialidase inhibition assay and antibody responses to certain heat shock proteins can serve as sensitive markers for previous *T. cruzi* infection in patients who are parasite free and serologically cured (91). ELISA procedures have also been used to detect specific antibodies in naturally infected dogs (92).

Although Bolivian donor screening studies using indirect hemagglutination tests, indirect fluorescent-antibody tests, and four different ELISAs demonstrated 96.5 to 100% sensitivity and 87.0 to 98.9% specificity, use of a single test results in unacceptable numbers of false-negative samples in areas of highly endemic infection or in at-risk populations. Use of two tests would miss only 1 infected unit per 10,000 screened; however, the selection of tests depends on costs and feasibility (93).

The common occurrence of inconclusive results in blood donor screening for anti-*T. cruzi* antibodies should be interpreted with caution. Blood transfusion services in Brazil and some other Latin America countries simultaneously perform two tests that use different methodologies to detect anti-*T. cruzi* antibodies; this is determined by legislation currently in force in Brazil. Unfortunately, total understanding of the results of low reactivity screening tests has not been achieved; thus serology screening information must be considered in association with epidemiological data (94–97). The use of riboflavin and UV light to reduce the levels of infectious *T. cruzi* in whole blood could be effective in preventing transfusion of these parasites (98). Currently, it is estimated there may be as many as 300,000 individuals with Chagas' disease within the United States, almost all of whom involve people moving into the U.S.

KEY POINTS—LABORATORY DIAGNOSIS

Trypanosoma cruzi

1. Laboratory workers should use blood-borne-pathogen precautions when examining blood from Chagas' disease patients, because the trypomastigotes are infective.
2. Trypomastigotes are prevalent in the blood of patients with acute Chagas' disease; however, organism numbers are much smaller in the indeterminate and chronic stages of the infection.
3. *T. rangeli* cannot be differentiated from *T. cruzi* on the basis of parasite morphology; patient information regarding geographic exposure is required for more appropriate interpretation of laboratory results.
4. In addition to thin and thick blood smears, concentration methods should be used to concentrate the trypomastigotes in the blood.
5. Immunoassays for antigen detection are now available and are highly sensitive and specific. Alternative methods such as culture and serologic testing can be used; however, these approaches may not be feasible without the use of a reference laboratory. Molecular methods are also being more widely used.

Treatment

Although numerous drugs have been tried, including those used to treat African trypanosomiasis and leishmaniasis, few have proven to be effective for therapy of Chagas' disease. In acute and congenital Chagas' disease and infections caused by laboratory accidents, treatment should be administered as soon as possible, even though in some cases symptoms are self-limited. Drug therapy has little effect on reducing the progression of chronic Chagas' disease.

Benznidazole (RO-7-1051, Rochagan, Radanil), an imidazole derivative, is effective in reducing or suppressing parasites in the acute stages of disease but has limited capacity to produce a parasitic cure. It appears to be slightly more active and better tolerated than nifurtimox and is currently the drug of choice (32, 99). Benznidazole is taken orally for prolonged periods and, similarly to nifurtimox, has little effect on changing clinical manifestations or reducing the progression of chronic Chagas' disease. Side effects include peripheral polyneuropathy, abdominal pain, nausea, vomiting, and severe skin reactions. The drug should be taken with meals to minimize gastrointestinal adverse effects and is contraindicated during pregnancy (32).

Nifurtimox (Lampit), a nitrofurfurylidine derivative, is tolerated better in younger than older patients and should not be used during pregnancy. It reduces the duration and severity of illness and decreases mortality due to acute and congenital Chagas' disease. Reversible gastrointestinal, cutaneous, and neurologic adverse effects are common. Treatment success varies from one country to another, possibly indicating differences in the susceptibility of strains of *T. cruzi*. There is no indication that treatment of patients with chronic Chagas' disease is beneficial. Nifurtimox must be taken orally for prolonged periods, and there can be severe side effects including abdominal pain, nausea, vomiting, anorexia, and neurologic symptoms.

Allopurinol, a purine analog, was found in limited clinical trials to be as effective as nifurtimox and benznidazole in treating Chagas' disease. The drug is taken orally, and its side effects include skin rashes in patients with renal impairment, epigastric pain, transient diarrhea, and pruritus. In a study using itraconazole or allopurinol in patients with chronic Chagas' disease, parasitologic cure was evident in 44% of those treated with allopurinol and 53% of those treated with itraconazole. Electrocardiographic evaluation showed normalization in 36.5 and 48.2%, respectively, of patients with chronic or recent cardiopathy (100).

Symptoms associated with megaesophagus and megacolon may be treated with dietary measures or may require surgery. Patients with chronic chagasic heart disease may receive supportive therapy or be managed in some cases with pacemakers.

Epidemiology and Prevention

Epidemiology. Chagas' disease is a zoonosis occurring throughout American continents and involves reduviid bugs living in close association with human reservoirs (dogs, cats, armadillos, opossums, raccoons, and rodents) (Table 28.5) (44, 101). The most ubiquitous sylvatic reservoir host is the opossum, *Didelphus* sp., which is found throughout much of the range of *T. cruzi* in the Americas. Multiple nesting or resting sites of the opossum encompass many types of triatomine habitat. High *T. cruzi* prevalence rates are partly due to the fact that opossums eat triatomines and may also transmit infection via anal gland secretions. Sylvatic cycles of *T. cruzi* transmission extend from southern Argentina and Chile to northern California. Housing conditions are extremely important in transmission; the prevalence and incidence of infection are very high in human dwellings where the vector has adapted to living in the mud and in thatch walls and palm leaf roofs.

The reduviid bug has easy access to humans to obtain blood meals and transmit the infection in this type of dwelling. A significant reduction in transmission was noted to occur in houses with walls made of plaster where cracks and crevices were covered, in contrast to houses with mud or thatch walls. Human infections occur mainly in rural areas where poor sanitary and socioeconomic conditions and poor housing provide excellent breeding places for reduviid bugs. These conditions allow maximum contact between the vector and humans. Although 12 species of reduviids occur within the United States, they have not adapted themselves to household habitation (102). Humans should avoid sleeping in thatch, mud, or adobe houses; bed nets should be used by persons sleeping in these types of houses. Travelers planning to stay in hotels, resorts, or other well-constructed housing facilities are not at high risk for contracting Chagas' disease. Insecticides can be used to kill the vectors and reduce the risk of transmission. Also, one must remember that in some countries, the blood supply may not always be screened for Chagas' disease and so blood transfusions may carry a risk of infection. The severity of Chagas' disease varies with the geographic area and may be related to strain differences in *T. cruzi*.

Transmission to humans is highly dependent on the defecation habits of the insect vector. In areas where the local species of reduviid bug does not ordinarily defecate while feeding, there are no human infections. This may explain why there are few human infections originating in the United States, even though sylvatic infections are known to occur in southern states. A number of autochthonous cases have been reported in the United States, in Texas, California, and the Southwest (103, 104). The reduviid species involved in transmitting the infection to humans vary with the geographic area. Various species involved include *Panstrongylus megistus*, *Rhodnius prolixus*, *Triatoma brasiliensis*, *T. infestans*, *T. protracta*, *T. guasayana*, and *T. sordida* (Table 28.6).

Approximately 90 million people within the United States participate in wildlife activities, thus increasing their potential exposure to the *T. cruzi* vector. Another possible exposure mechanism could be direct contact of hunters with infectious blood and tissues of wild animal reservoirs. A study of healthy people who were discovered via blood donor screening to be positive for Chagas' disease revealed the surprising observation that 6 of 17 (36%) acquired their infection in the U.S. rather than endemic Latin America. Also, most individuals (67%) reported hunting and camping activities over a 10+-year period. Therefore, time spent in outdoor activities in rural settings certainly appears to increase the chances for exposure to the vector and/or the parasite itself (110).

Several outbreaks with acute Chagas' disease in the Brazilian Amazon basin have been linked to the consumption of Amazonian palm berry or açaí juice, probably contaminated with triatomid bug feces on the fruit or with bugs that were accidentally crushed during the preparation process. Without subsequent pasteurization, the organisms remain viable, especially if the juice is not frozen. Although these outbreaks occurred in 2005 and 2007, reports from the 1960s were highly suggestive for food transmission of *T. cruzi* in Brazil (111).

Prevention and Control. Until recently, control of Chagas' disease has been mainly through the use of insecticides to eliminate the reduviid vector. In certain areas, insecticide resistance in triatomids has been noted. In addition to residual insecticide-spraying programs, construction of reduviid-proof dwellings and education are essential for effective control programs. Improvements in unsanitary living conditions, plastering of walls to obtain a smooth, crack-free surface, and replacement of palm-thatched roofs with metal roofing have been shown to considerably reduce the number of reduviids in houses. Although control of Chagas' disease is feasible, few countries have initiated control programs because of both political and economic constraints.

Transmission can occur through organ transplants and blood transfusion (105). Some of the countries in areas where the disease is endemic have laws mandating serologic testing of blood donors. Financial constraints may

TABLE 28.6 *Trypanosoma cruzi*: principal vectors

Species	Geographic area
Panstrongylus megistus	Eastern seaboard of Brazil
Rhodnius prolixus	Venezuela, Colombia, French Guiana, Guyana, Suriname; most important vector in northern South America and Central America
Triatoma brasiliensis	Central and eastern Brazil
T. dimidiata	Ecuador, Colombia, western Central America, Mexico
T. gerstaeckeri	Arizona, Texas, southern United States, Mexico
T. indictiva	Arizona, Texas
T. infestans	Brazil, Bolivia, Peru, Chile, Argentina, Paraguay, Uruguay
T. lectularia	Arizona
T. protracta	Arizona, Texas, California
T. recurva	Arizona
T. rubida	Arizona, California
T. sanguisuga	Arizona, Texas, southeastern United States
T. sordida	Brazil, Argentina, Bolivia, Paraguay, Uruguay; often associated with chickens
T. guasayana	Argentina

hinder the implementation of these laws, and there is a lack of standardization of currently available serologic tests. An alternative approach to serologic screening in the United States is the use of a questionnaire to identify prospective donors who may have resided in high-risk areas. This approach may not be practical, since even in vector-free areas in countries with endemic infection a significant portion of the donor blood units were positive for antibodies to Chagas' disease. In areas where seroprevalence is high, rather than discarding all positive blood units, laboratorians add gentian violet to the units and store them at 4°C for 24 h before use to kill the organism.

Evaluation of electrocardiographic (ECG) changes among urban workers in Sao Paulo, Brazil, showed that there were 2.2% positive sera for *T. cruzi* among 27,081 workers. A much higher percentage of workers with ECG abnormalities (42.7%) were seropositive for *T. cruzi* compared with the percentage of workers with ECG abnormalities (19.8%) who were seronegative. Based on these data, it appears that the high frequency of ECG abnormalities emphasizes the importance of providing medical assistance to this group (106).

Chagas' disease has been identified as an important emerging infection within the United States. In a pilot study from 2007 to 2012 of blood donors in Texas who had tested positive for Chagas' disease, those with confirmed infection were investigated for (i) infection source, cardiac symptoms, and health comorbidities, (ii) electrocardiography, (iii) echocardiography if required, and (iv) measurement of a highly sensitive troponin T biomarker. In participants with confirmed infection, 41% (7 of 17) had electrocardiographic abnormalities consistent with Chagas' cardiomyopathy. Also, 36% (6 of 17) were thought to have acquired the infection locally. Troponin T serum levels increased with cardiac severity. Based on these findings, the authors strongly recommended that cardiologists consider Chagas' disease in patients with electrocardiographic changes or cardiomyopathy consistent with Chagas' disease, even if there is no history of living in areas of endemicity (112).

Laboratory personnel working with *T. cruzi* must be aware of the hazard involved in handling the highly infectious trypomastigote form. Precautions would include the use of gloves and eye protection. All clinical specimens must be handled by using standard precautions.

Another potential source of infection involves veterinarians who may be handling dogs with *T. cruzi* infections, all of which may be infectious for humans coming in contact with the canine blood.

Trypanosoma rangeli

T. rangeli was first described by Tejera during an examination of intestinal contents from the reduviid *R. prolixus*. The infection in human blood was first found by DeLeon in Guatemalan children. *T. rangeli* infections are encountered in many areas where *T. cruzi* also occurs; consequently, the two parasites may be confused and must be differentiated morphologically (Table 28.5). No pathology is associated with *T. rangeli* infections. In a study conducted in Venezuela, the relatively high frequency of *T. rangeli* infections detected in patients with acute-stage Chagas' disease (18.6%) complicated the diagnosis of *T. cruzi* infection. More than 2,600 cases of human infection have been documented in Venezuela, Guatemala, Panama, Colombia, El Salvador, Costa Rica, Peru, and Brazil, the majority of which have been in Venezuela and Guatemala (107).

Life Cycle and Morphology

After the reduviid bug is infected during a blood meal, *T. rangeli* migrates from the intestine to the hemolymph and then to the salivary glands, where infective metacyclic forms can be found (Fig. 28.23). Transmission to humans is through the bite of the reduviid (triatomid) bug *R. prolixus* and related species by inoculation of metacyclic try-

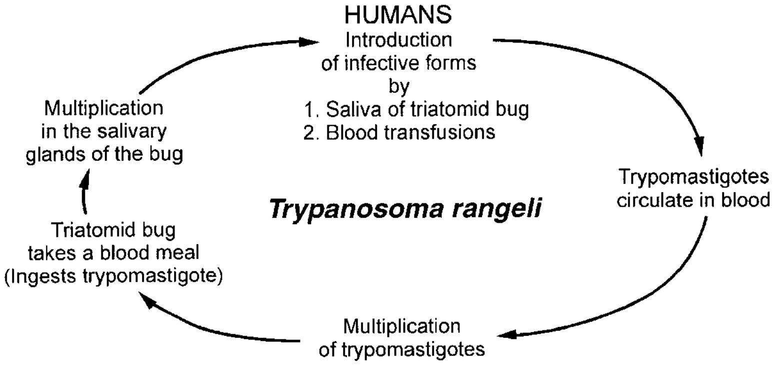

HUMANS
Introduction of infective forms by
1. Saliva of triatomid bug
2. Blood transfusions
Trypomastigotes circulate in blood
Multiplication of trypomastigotes
Triatomid bug takes a blood meal (Ingests trypomastigote)
Multiplication in the salivary glands of the bug
Trypanosoma rangeli

Figure 28.23 Life cycle of *Trypanosoma rangeli*. doi:10.1128/9781555819002.ch28.f23

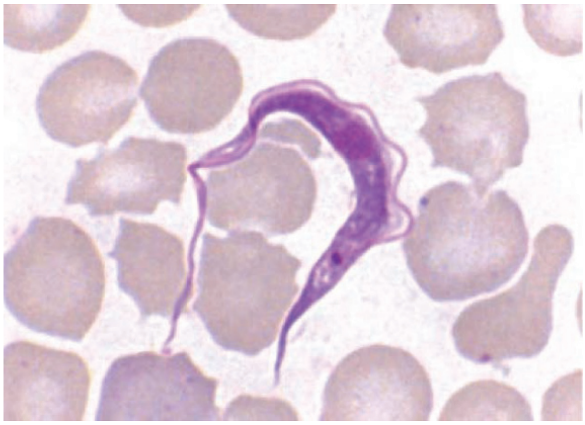


Figure 28.24 *Trypanosoma rangeli* trypomastigote in a peripheral blood smear. Note the small kinetoplast. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch28.f24

pomastigotes into the wound from the infected saliva. Infections with *T. rangeli* and *T. cruzi* can coexist in the reduviid vector. The trypomastigote is approximately 30 µm long and has a free flagellum (Fig. 28.24). The nucleus is anterior to the middle of the body, and the small kinetoplast is subterminal.

Clinical Disease

Human infections are apparently asymptomatic, and trypomastigotes have been noted in the blood for longer than a year. No evidence of pathogenicity has been detected in human volunteers (108).

Diagnosis

Trypomastigotes can be detected in the blood of infected patients by using thin and thick blood smears and buffy coat concentration techniques (Table 28.5). The parasites can be stained with any of the blood stains. Infections can also be detected by xenodiagnosis, in which the reduviid vector is allowed to obtain a blood meal from an infected patient and then the hemolymph and salivary glands are examined for epimastigotes and metacyclic trypomastigotes. In addition, blood can be cultured (Tobie's medium and Novy-MacNeal-Nicolle medium) or injected into laboratory animals (mice), then examined for epimastigotes and trypomastigotes, respectively. Although there are no serologic tests for *T. rangeli*, serologic cross-reactions have been noted to occur with tests for *T. cruzi*. A 48-kDa protein has been identified that is a specific and abundant intracellular antigen for *T. rangeli* epimastigotes. Results suggest that this antigen may be a useful marker for the identification and characterization of *T. rangeli* isolates (109).

KEY POINTS—LABORATORY DIAGNOSIS

Trypanosoma rangeli

1. *T. rangeli* trypomastigotes can be detected in the peripheral blood by using thin and thick blood smears and concentration techniques; however, in practice *T. rangeli* is rarely seen in human blood.
2. *T. rangeli* trypomastigotes are morphologically similar to African trypanosome trypomastigotes (small kinetoplast).
3. *T. rangeli* infections can occur together with *T. cruzi* infections; therefore, *T. rangeli* trypomastigotes must be differentiated from trypomastigotes of *T. cruzi* in the peripheral blood.

Epidemiology and Prevention

T. rangeli infections have been found in both Central and South America and often overlap with *T. cruzi* infections. In some areas, *T. rangeli* infections are five to six times more frequent than *T. cruzi* infections in the population.

Animals naturally infected with *T. rangeli* include opossums, rodents, anteaters, raccoons, dogs, and nonhuman primates. The organisms appear to be harmless to animals; however, they do reduce the life span of the reduviid vector (108).

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29

Unusual Parasitic Infections

Aquatic protist

Rhinosporidium seeberi

Protozoa

Lophomonas blattarum
Dictyostelium polycephalum
Myxozoan parasites
Trypanosoma evansi, *Trypanosoma lewisi*

Nematodes (the roundworms)

Ancylostoma ceylanicum
Halicephalobus gingivalis
Oesophagostomum spp.
Eustrongylides spp.
Mermis nigrescens
Dioctophyma renale
Ternidens deminutus
Mammomonogamus laryngeus (*Syngamus laryngeus*)
Ascaris suum
Gongylonema pulchrum
Haycocknema perplexum

Cestodes

Diplogonoporus spp.
Bertiella studeri
Inermicapsifer madagascariensis
Raillietina celebensis
Mesocestoides spp.
Taenia crassiceps

Trematodes

Alaria americana
Plagiorchis spp.
Neodiplostomum seoulense
Spelotrema brevicaeca
Brachylaima sp.
Nanophyetus (*Troglotrema*) *salmincola*
Stellantchasmus falcatus
Phaneropsolus spinicirrus, *Phaneropsolus bonnei*, and *Prosthodendrium molenkempi*
Haplorchis taichui
Gymnophalloides seoi
Metorchis conjunctus (North American liver fluke)
Schistosoma mattheei
Philophthalmus lacrimosus
Achillurbainia spp.

Pentastomids

Armillifer spp., *Linguatula serrata*, and *Sebekia* spp.
Acanthocephalans
Macracanthorhynchus hirudinaceus and *Moniliformis moniliformis*

Most parasites infecting humans are recognized as being able to survive easily within the human host and to possibly cause disease, but there are some organisms that only rarely infect humans. Perhaps humans are only accidental hosts. In any case, because these infections, being so rare, are not reported frequently, such parasites may not be considered possible etiologic agents. It is important to at least be aware of such parasites and of the possible disease process created by these relatively benign organisms, because they can become a problem in immunocompromised patients. Examples of these unusual infections are summarized in Table 29.1.

Aquatic Protist

Rhinosporidium seeberi

Rhinosporidiosis is a granulomatous disease of humans and animals that is characterized by slow-growing tumorlike polyps, affecting primarily the nasal mucosa and ocular conjunctiva. The causative agent is *Rhinosporidium seeberi*, an organism that has undergone several reclassifications. Although it was thought to be a fungus or protozoan, it is now placed in a novel clade of aquatic protistan parasites, the Mezomycetozoea; this occurs at the divergence between animals and fungi (1, 2). Since this organism cannot be grown in synthetic media or human or animal cell lines, diagnosis is established by seeing the organism in tissue biopsy specimens. Two stages are seen in tissue: the larger endosporulating spherical form, or sporangia, and the smaller trophocyte (Fig. 29.1). The genus *Rhinosporidium* may include multiple host-specific strains (3). Infection generally occurs after swimming in stagnant freshwater ponds, lakes, or rivers, but is also suspected to occur from dust or air.

The sporangium is the mature form and measures 100 to 350 µm; contained within the sporangium are numerous endospores (sporangiospores). The zonal arrangement of sporangiospores is characteristic, with the immature ones forming a crescent-shaped mass at the periphery, the maturing ones arranged sequentially toward the center, and the mature ones in the center. This arrangement of the sporangiospores separates *R. seeberi* from other spherical endosporulating organisms seen in tissue.

Rhinosporidiosis has been found in young men between 20 and 40 years old; the lesions tend to be slow-growing polyps or tumor-like masses, usually

doi:10.1128/9781555819002.ch29

TABLE 29.1 Unusual parasitic infections

Organism	Body site	Diagnosis	Comments
Aquatic protist			
Rhinosporidium seeberi	Nasopharynx, ocular conjunctiva, genitalia	Identification of sporangium and/or trophocytes in tissue biopsy specimens	Cannot be grown in synthetic media or animal/human tissue cell lines; classification has been difficult over a number of years
Protozoa			
Lophomonas blattarum	Bronchopulmonary disease	Recovery of flagellate in pulmonary specimens	Can easily be confused with ciliated epithelial cells; metronidazole
Dictyostelium polycephalum	Eye, keratitis	Clinical picture identical to that caused by *Acanthamoeba*	Growth on nonnutrient agar plate with *Escherichia coli* overlay
Myxozoa: *Myxobolus* spp.	Intestine	Recovery of spores in stool	The morphology can mimic normal human spermatozoa, causing confusion with possible sexual abuse situations; true human infection is still somewhat unclear
Trypanosoma evansi, *Trypanosoma lewisi*	Blood	Thick and thin stained blood films	Molecular methods may be required to definitively identify the causative organism
Nematodes			
Ancylostoma ceylanicum	Intestine; mimics clinical picture seen in normal hookworm infections	Eggs in the stool; eggs resemble those of other hookworms, peripheral eosinophilia	Primarily seen as infections in dogs and cats; second most common hookworm infecting humans
Halicephalobus gingivalis (formerly *Micronema deletrix* or *H. deletrix*)	Brain; meningoencephalitis	Routine histologic test with consultation	Free-living worms living in soil, manure, humus; occasionally parasitize horses
Oesophagostomum spp.	Intestine or abdominal cavity (nodular mass lesions)	Examination of nodule (dissection or aspirate); definitive diagnosis depends on identification of worm (may be difficult)	Carcinoma, tuberculosis, or Crohn's disease often suspected; mass often attached to bowel wall or ileum or colon; infection via ingestion of larvae
Eustrongylides spp.	Abdominal cavity	Identification of larvae (bright red)	Rare but may increase with consumption of raw fish; infective larval form also found in amphibians and reptiles
Mermis nigrescens	Mouth	Identification of larvae or adults	Soil nematodes with eggs deposited on plants and larvae in grasshoppers; adult worm accidentally transmitted to infant, probably by dog or cat
Dioctophyma renale	Primarily right kidney (may be in left kidney or body cavities)	Identification of worm in autopsy specimens or living worms passed from the urethra	Route of infection probably ingestion of raw freshwater fish and frogs; widely reported in dogs
Ternidens deminutus	Large intestine	Identification of eggs in stool; ulcers and/or cystic nodules may be seen in the large intestine	Third-stage larvae will develop in soil; probably infective for humans; life cycle not known
Mammomonogamus laryngeus	Trachea	Identification of Y-shaped male and female worms (*in copulo*) from coughed-up sputum or from eggs in sputum or stool	Third-stage larvae thought to be infective for the vertebrate host; normal host cattle, felines; life cycle not known
Ascaris suum	Intestine or liver, lungs; intestine but worms do not mature (can be serious disease)	Identification based on size and shape of denticles and patient history of exposure to pigs raised on farms; dose required for symptoms of pneumonitis very minimal; large doses can lead to severe disease	Ingestion of infective eggs probably more common than thought, particularly in people who live and/or work on farms where pigs are raised
Gongylonema pulchrum	Mucosa or submucosa of buccal cavity; esophageal mucosa	Identification based on worm recovery and egg morphology	Accidental ingestion of insect intermediate hosts, possibly cockroaches or other infected insects

(continued)

TABLE 29.1 *(continued)*

Organism	Body site	Diagnosis	Comments
Haycocknema perplexum	Skeletal muscle fibers	Identification of adults or larvae in muscles	Symptoms of progressive muscle weakness, eosinophilia, active polymyositis
Cestodes			
Diplogonoporus spp.	Intestine	Identification of proglottids or eggs (similar to those of *Diphyllobothrium latum*)	Ingestion of infected fish; usual parasites of whales
Bertiella spp.	Intestine	Identification of chains of proglottids or very characteristic eggs	Accidental ingestion of mites containing the infective form; most infections seen in children
Inermicapsifer madagascariensis	Intestine	Identification of proglottids, eggs, or egg packets in stool	Very difficult to differentiate from *Raillietina* spp.; patient is asymptomatic to mild symptoms; usually seen in children; infection probably from ingestion of arthropod vector (unknown)
Raillietina celebensis	Intestine	Identification of proglottids, eggs, or egg packets in stool	Intermediate hosts are ants (accidental ingestion)
Mesocestoides spp.	Intestine	Identification of proglottids in stool	Infection probably due to ingestion of raw meat of some animal (unknown) carrying the intermediate larval form in the tissue; usually found in children; symptoms minimal
Taenia crassiceps	Eye, subcutaneous paravertebral infiltrate (AIDS)	Identification of small, white, spherical masses (2–3 mm) of larval worms	Common parasite of the red fox; in animals, larval forms found under skin and in body cavities; human cases very rare but very serious
Trematodes			
Alaria americana	Disseminated throughout many tissues including eye, organs, central nervous system; can be very serious disease	Recovery of mesocercariae at surgery or autopsy	Ingestion of inadequately cooked frogs or frog legs
Plagiorchis spp.	Intestine	Recovery of adult worms and/or eggs in stool	Ingestion of insect grubs containing the infective stage; raw fish also implicated in transmission
Neodiplostomum seoulense	Intestine	Recovery of adult worms and/or eggs in stool	Associated with ingestion of raw or poorly cooked snake meat, frogs, or house rats
Spelotrema brevicaeca	Intestine or disseminated to other tissues	Recovery of adult worms in stool and/or eggs in stool or tissues	Ingestion of raw crabs containing infective metacercariae; reported to cause severe illness in compromised patients
Brachylaima sp.	Intestine	Recovery of adult worms and/or eggs in stool	Ingestion of raw snails containing infective metacercariae; may be mild abdominal pain and diarrhea
Nanophyetus (Troglotrema) salmincola	Intestine	Recovery of adult worms and/or eggs in stool	Ingestion of raw fish viscera and/or giant salamander; rickettsia associated with infection causes severe illness in dogs, may also be human pathogen
Stellantchasmus falcatus	Intestine	Recovery of adult worms and/or eggs in stool	Ingestion of raw brackish-water fish; reported from Hawaii after ingestion of raw mullet
Phaneropsolus spinicirrus, *Phaneropsolus bonnei*, *Prosthodendrium molenkempi*	Intestine	Recovery of adult worms and/or eggs in stool; eggs resemble those of *Opisthorchis viverrini* or each other	Ingestion of raw small fish infected with naiads (water nymphs of dragonflies)

(continued)

TABLE 29.1 Unusual parasitic infections *(continued)*

Organism	Body site	Diagnosis	Comments
Haplorchis taichui	Small intestine	Identification of eggs or adult worms	Musosal ulceration, mucosal and submucosal hemorrhages, fusion and shortening of the villi, chronic inflammation, and fibrosis of the submucosa
Gymnophalloides seoi	Intestine	Recovery of adult worms and/or eggs in stool	New human intestinal trematode; southwestern island of Korea; bivalves serve as first or second intermediate hosts; eggs smaller than *Clonorchis* eggs
Metorchis conjunctus (North American liver fluke)	Biliary tree	Recovery of adult flukes and/or eggs in stool	Ingestion of raw white sucker fish (sashimi) containing infective metacercariae
Schistosoma mattheei	Venules of the bladder and/or intestine	Recovery of eggs in urine and/or stool; eggs resemble those of *S. intercalatum*	Skin penetration of infective cercaria from freshwater snail; *S. mattheei* and *S haematobium* may evolve into a single species
Philophthalmus lacrimosus	Eye, conjunctivitis	Sensation of having a foreign body in the eye	Identification of adult worm, generally from the eye, rare
Achillurbainia spp.	Granulomata, retroauricular cysts or abscesses	Identification of eggs or adult worms from biopsy specimens	Symptoms may or may not be present
Pentastomids (tongue worms)			
Armillifer spp., *Linguatula serrate*, *Sebekia* spp.	Infective larvae die *in situ* (tissues, including the eye); ingestion of mature larvae may lead to development of adults in nasopharyngeal tissues (halzoun syndrome)	Identification of the worm/larvae in biopsy or autopsy specimen	Most infections acquired from ingestion of raw, infected snakes; liver, lung, spleen involved; often not discovered until autopsy; lung involvement may cause congestion
Sebekia spp.	Dermatitis, serpiginous burrow, erythema	Larvae in burrow	Adult parasites of respiratory tract of reptiles, immature forms found in viscera, muscles, along spinal cord of freshwater fish
Acanthocephalans			
Macracanthorhynchus hirudinaceus	Intestine	Identification of adult worms; immature worms very difficult to identify	Infections acquired by ingestion of raw beetles; clinical manifestations are intestinal obstruction and perforation; eggs often not seen in feces
Moniliformis moniliformis	Intestine	Identification of adult worms; immature worms very difficult to identify; eggs can be used to identify the helminth	Infections acquired by ingestion of raw cockroaches; symptoms can be diarrhea, pallor, anorexia, weight loss, weakness

of the nasal mucosa or conjunctiva. Lesions have been found in the paranasal sinuses, larynx, and external genitalia. Dissemination appears to be rare. Symptoms include nasal obstruction and epistaxis. Surgical excision of the lesions is generally recommended as the treatment of choice; if total excision is not possible, recurrences are typical. As an ocular disease, it often presents as a benign conjunctival tumor but may mimic other ocular conditions.

Over 90% of rhinosporidiosis cases occur primarily in India and Sri Lanka; however, cases have been reported from the Americas, Europe, Africa, and Asia. Three cases have been reported from the southeastern United States. Various studies have linked infection to swimming or bathing in freshwater ponds, lakes, or rivers (1–3).

Protozoa

Lophomonas blattarum

The natural habitat of the multiflagellate protozoon *Lophomonas blattarum* is as an endocommensal in the hindgut of insects such as cockroaches. However, it also causes bronchopulmonary disease in humans. A total of 61 cases have been identified in the literature. Most of the reports were from China, with some cases from Peru and Spain. Although most cases were seen in adult males, some pediatric cases were reported from Peru. Clinical presentation was nonspecific, including symptoms and complaints such as fever, cough, and being out of breath. This protozoan appears as a more round structure (20 to 25 μm in diameter) and contains coarse cytoplasmatic granules and some vacuoles, with a tuft of wavy flagella which are longer and more varied in length, occurring at one end of the cell, and without a terminal bar (Fig. 29.2). Bronchial ciliated epithelial cells include the following features: a columnar cellular shape; a clear nucleus at the end of the cytoplasm; fine nuclear chromatin; clearly delineated thin nuclear membrane; and cilia that are straight, combed, of uniform length, at the apex of the cell, and inserted into a terminal bar (4, 5). Therapeutic response with metronidazole has been documented.

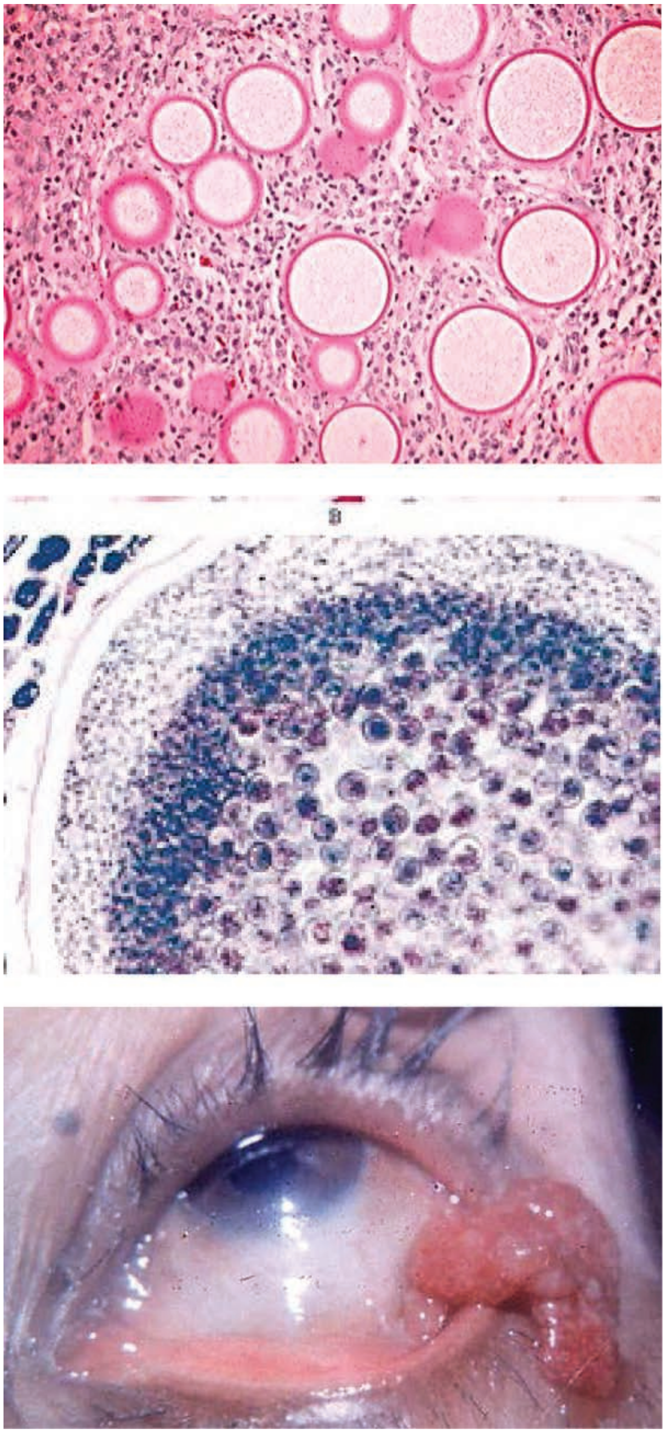
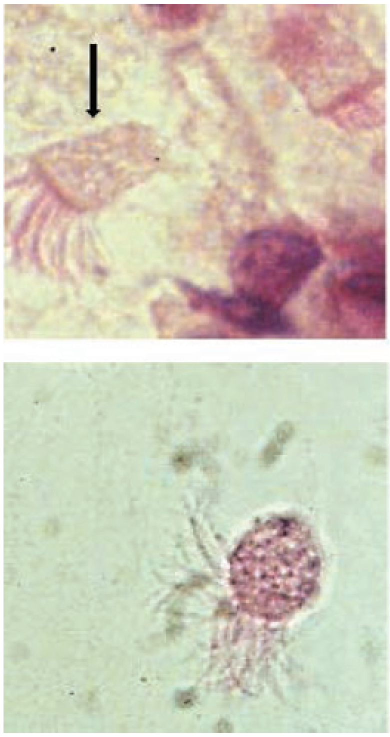


Figure 29.1 Histology of rhinosporidiosis; human nasal polyp. (Top) Immature *Rhinosporidium seeberi* trophocytes; note the thick walls and the surrounding inflammatory cells (from the Centers for Disease Control and Prevention). (Middle) Sporangium containing endospores (from reference 2, with permission). (Bottom) Rhinosporidiosis of the conjunctiva (courtesy of Doctorfungus Corporation, J. Moses, 2000).
doi:10.1128/9781555819002.ch29.f1

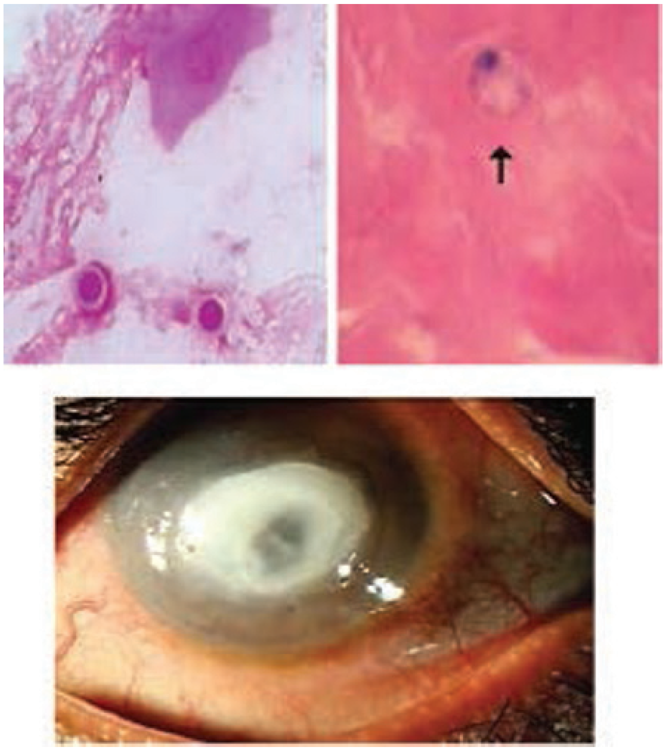
Figure 29.2 Morphologic differences between cytoplasmic fragments of ciliated bronchial cells (arrow) (top) and the flagellate protozoon *Lophomonas blattarum* (bottom) in stained sputum smears. (Courtesy of Rafael Martinez-Giron, Fundacion INCLINICA, Spain; cover image from *J Med Micro*, Nov. 2013, volume 62; reference 4.) doi:10.1128/9781555819002.ch29.f2



Dictyostelium polycephalum

Members of the genus *Dictyostelium* (social amebas or cellular slime molds) are divided into four high-level taxa with several species on the basis of DNA phylogeny. The life cycle of *Dictyostelium* spp. consists of an ameboid vegetative phase, a cyst phase, and a plant-like fruiting phase. In culture, it grows at a temperature of 34 to 35°C, which is higher than that for other species of *Dictyostelium*. Most myxamoebae aggregate to form sporocarps; however, some may round up in individual cells to form microcysts. The *D. polycephalum* isolated from a patient with keratitis grew at 36°C on nonnutrient agar with an *Escherichia coli* overlay. After 48 hours of incubation, a nonnutrient agar plate showed growth of double-walled, spherical cysts 6 to 7 µm in diameter that had different morphologic features than those of *Acanthamoeba* spp. cysts (Fig. 29.3). The clinical picture for keratitis caused by *D. polycephalum* tends to be indistinguishable from that caused by *Acanthamoeba* spp. Based on DNA extraction and PCR, the organism was confirmed as *D. polycephalum* (6). Infection with this organism could have come from contaminated water or soil. Treatment is the same for this infection as for *Acanthamoeba* keratitis; in serious infections, keratoplasty is required.

Figure 29.3 *Dictyostelium polycephalum*. (Upper, left) Spherical double wall cysts from culture (Gram stain); (right) corneal button showing cysts (arrow), hematoxylin and eosin stain. (Lower) Cornea of eye showing ring-shaped central infiltrate and central thinning (very similar to that seen with *Acanthamoeba* infections) (courtesy of reference 6, with permission.)
doi:10.1128/9781555819002.ch29.f3



Myxozoan Parasites

Myxozoans (*Myxobolus* spp.) are commonly found as parasites in aquatic vertebrates (especially fish) and invertebrates. They are recognized as a separate phylum with two classes: the Myxosporea, which infect fish, amphibians, and reptiles, and the Actinosporea, which infect aquatic invertebrates (primarily oligochaetes and sipunculids) (7–9). Classification has been difficult, and it has been recommended that a number of factors be used in making taxonomic decisions, including determination of 18S rRNA gene sequences combined with information on tissue tropism, host species infected, and developmental cycles in the fish and alternate host (when known). There are over 1,000 species, and new ones are being described each year; however, many of the life cycles have not yet been defined. Alternation between actinosporean stages in oligochaetes and myxosporean stages in fish may occur (10). These organisms parasitize a variety of fish tissues, where they produce pseudocysts that contain hundreds of thousands of small spores. As marine netpen farming of salmonid fish continues to expand in many countries, more unusual host-parasite relationships may be seen in the future (11).

Many of these myxozoan spores contain chitin in their spore coat, and the spores can be passed intact through the vertebrate gastrointestinal tract (12). There is no evidence that these organisms cause human disease; however, spores have been recovered in stool samples from patients with gastrointestinal tract symptoms, including abdominal pain and/or diarrhea (13). There is also speculation that these parasites could be potentially infectious and pathogenic in immunocompromised patients. Coinfections with other parasites such as *Cryptosporidium* spp., *Cystoisospora belli*, *Giardia lamblia*, *Hymenolepis nana*, *Strongyloides stercoralis*, and *Ascaris lumbricoides* have been found, thus complicating the determination of the pathogenic role of *Myxobolus* spp.

Spores of a myxosporean parasite (*Henneguya salminicola*) have been detected in fecal samples from two human patients (7), in one case being mistaken for human spermatozoa and leading to suspicion of sexual abuse (Fig. 29.4). The spores were passed undigested in feces after the consumption of infected salmon and were not thought to have any causal relationship to the patient's symptoms. True infections with myxosporean parasites in humans have not previously been recorded, other than this case. Organisms resembling human spermatozoa have been seen in fecal

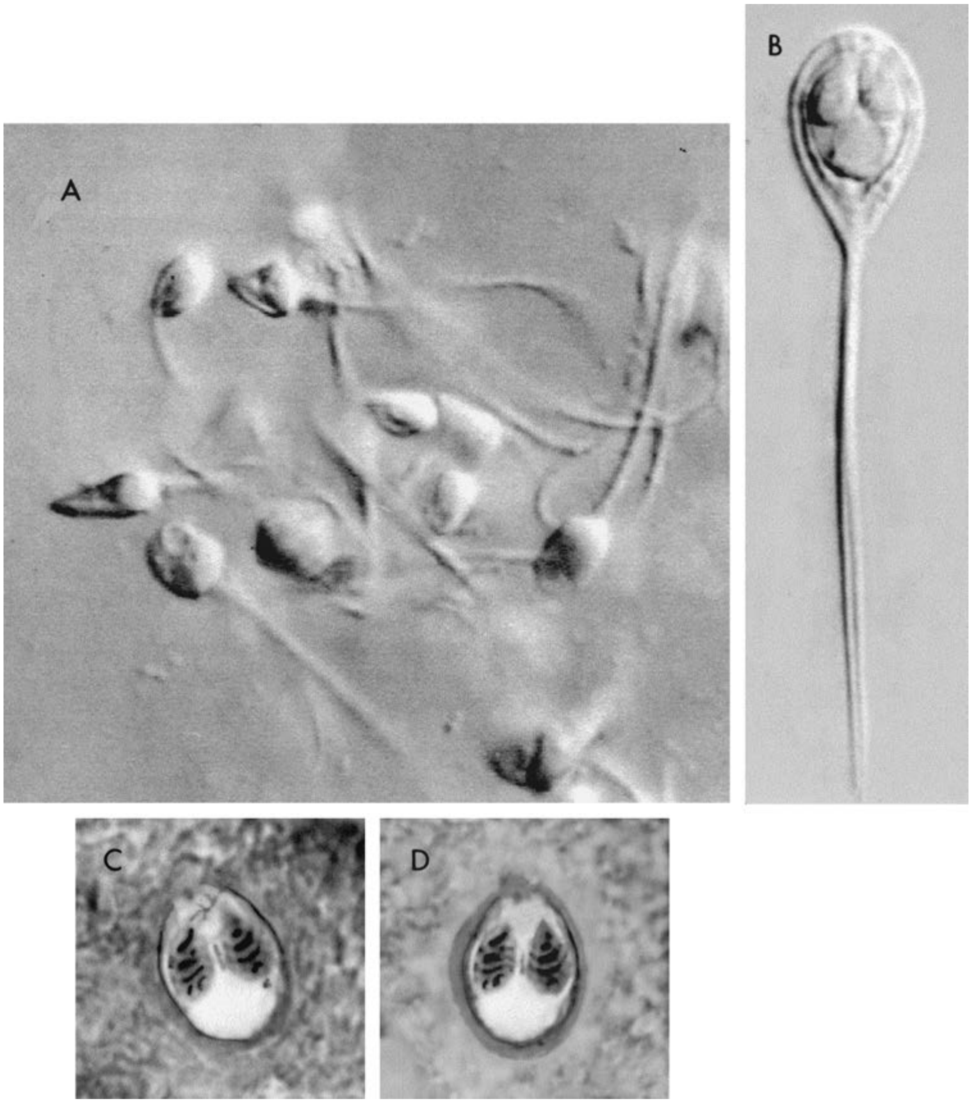


Figure 29.4 (A) Normal human spermatozoa. (B) Myxosporean spore from salmon. Note the similarities between panels A and B. (C and D) Two spores demonstrating the polar filaments. (Reprinted from reference 7 with permission.)
doi:10.1128/9781555819002.ch29.f4

concentrates at high dry magnification (×400). They measured about 40 µm in total length with a teardrop-shaped spore body of approximately 10 µm, which tapered gradually to one or two caudal filaments. Most of the spores had two pyriform polar capsules situated in the anterior region of the spore body. Although routine iron hematoxylin and trichrome staining failed to stain the spores, the modified trichrome stain was effective.

A second report discussed the detection of a second myxosporean species in stool samples from three additional patients (13). It is very likely that additional cases will be recorded; it is important that clinicians and microbiologists understand the possible presence of myxozoan spores in stool samples, particularly in patients with a high dietary intake of fish.

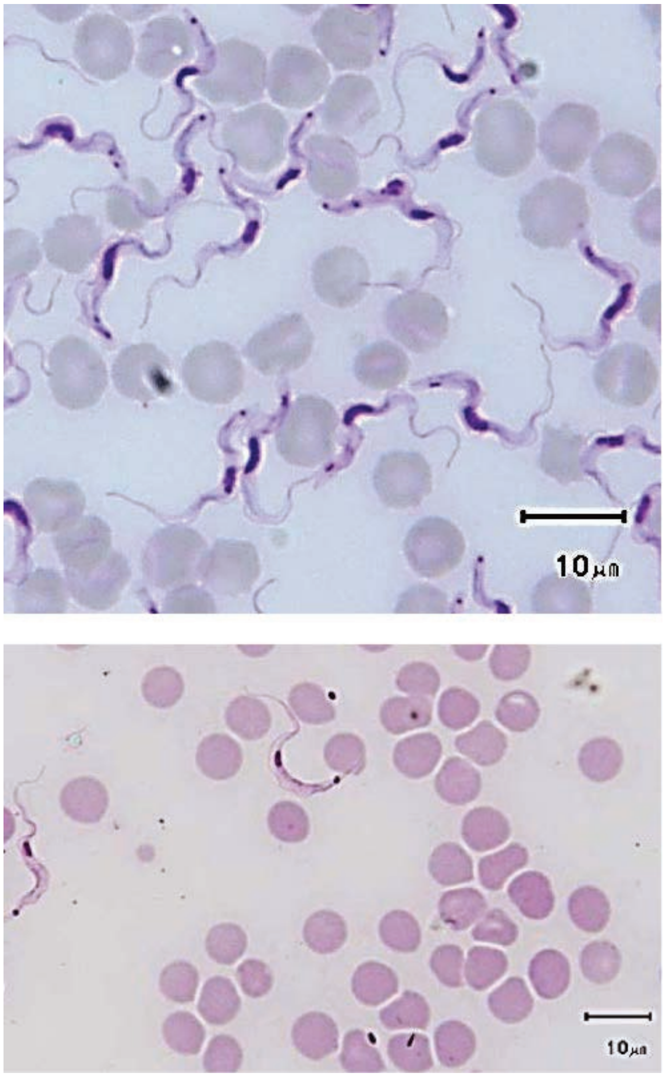
In an iodine preparation, the spores appear as dark pyriform bodies, while in modified acid-fast stains, the spore wall and enclosed sporoplasmic and capsulogenic cells stain acid fast. When the fecal specimen is stained with trichrome stain, the spores appear as darkly stained pear-shaped bodies measuring 11 to 12 µm by 7 to 8 µm with two polar capsules each measuring 5 by 2 µm. It is important to use the modified acid-fast stains, since these methods allow the visualization of the convolutions of the polar filament.

Trypanosoma evansi, Trypanosoma lewisi

T. evansi is responsible for a widely distributed disease called "surra" in domestic and wild animals found in Asia, Africa, South America, and even Europe. *T. lewisi* is a worldwide nonpathogenic parasite of rats transmitted by fleas. In recent years, *T. evansi* and *T. lewisi* have emerged as potentially pathogenic for humans (Fig. 29.5). While some of these cases reviewed herein were transient, six required trypanocidal treatments that were mostly successful, although two patients died.

In 1977, an accidental infection by a syringe containing *T. evansi*-infected blood was reported in India. In 1999, a human suspected of being infected with *T. evansi* was reported in Sri Lanka. He suffered from headache and episodes of hyperthermia consistent with high parasitemias. Unfortunately, the case was not thoroughly documented. In 2005, another case of *T. evansi* was reported in West Bengal State, India. The patient died 2 days after admission to a Kolkata hospital, but it is only suspected that death was caused by the related trypanosome infection. In 2010, in Egypt, a cattle farmer was infected with *T. evansi* and cured; however, this case remains doubtful because no attempts were made to ascertain the parasite species using molecular techniques and no clinical details or the drug used for treatment were given. Criteria such as the size and the position of the kinetoplast, the location of the nucleus, the development of the undulating membrane, the shape of the posterior end of the parasite, and the existence of a free flagellum may allow subgenus identification based on the morphological features of the parasites. However, species identification is not possible, and the parasitemia is sometimes too low for such observations to be conclusive. Consequently, molecular identification of trypanosomes is most often necessary. In 2004, the first molecularly confirmed case caused by *T. evansi* was described in Seoni, India, in a farmer showing a fluctuating trypanosome parasitemia associated with febrile episodes over the course of 5 months. The same signs were observed in a Sri Lankan patient in 1999.

Figure 29.5 (Upper) *Trypanosoma evansi* in a peripheral blood film. (Lower) *Trypanosoma lewisi* in a blood smear. (From reference 14, with permission, images courtesy of Marc Desquesnes.) doi:10.1128/9781555819002.ch29.f5



T. lewisi was found in the blood of three patients suffering from short febrile episodes in Malaysia and India. Infection was only transient and patients recovered without treatment. In 2006, trypanosomes were detected in blood of a 7-week-old baby living in Mumbai (India). She was suffering from fever and presented hepatosplenomegaly. Morphological examination of trypanosomes indicated *T. lewisi* or *T. lewisi*-like. The patient self-cured in 15 days. Several patients infected with *T. lewisi* had these infections confirmed using molecular methods (14).

Nematodes (the Roundworms)

Ancylostoma ceylanicum

Of the zoonotic hookworms, only *Ancylostoma ceylanicum* can develop to adulthood in humans; infections are found primarily in Asian countries and have been confirmed using molecular methods (15). These hookworms are endemic in Southeast Asia and are primarily seen as infections in dogs and cats (16). Recent molecular-based surveys in Asia, however, have demonstrated that *A. ceylanicum* is the second most common hookworm species infecting humans, comprising between 6% and 23% of total patent hookworm infections. *A. ceylanicum* infection mimics the clinical picture produced by normal hookworms in humans, including "ground itch" and moderate to severe abdominal pain in the acute phase (17). *A. ceylanicum* adults within the jejunum can produce chronic infections that produce anemia. In addition, the hookworm can act much like *Ancylostoma caninum* and be found lower in the gastrointestinal tract, leading to abdominal disten-

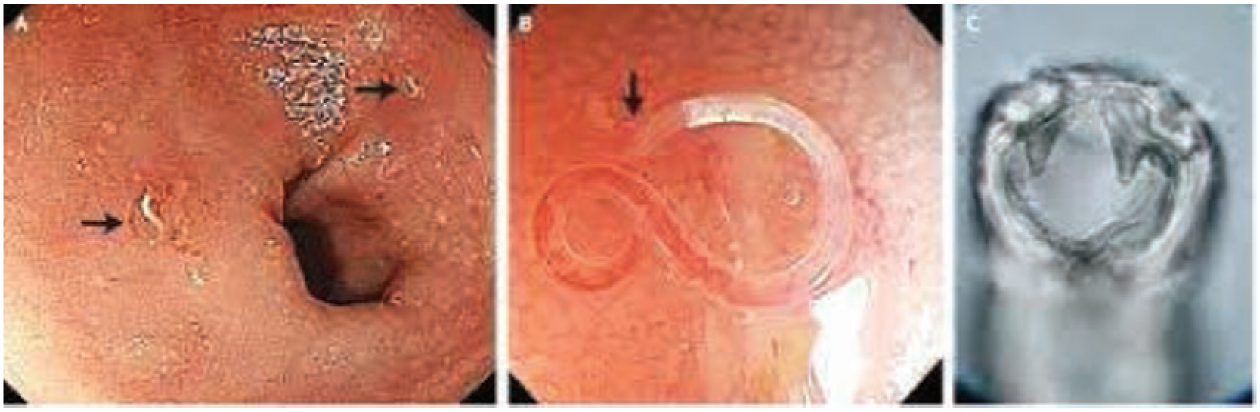


Figure 29.6 *Ancylostoma ceylanicum*. (A) Threadlike worms at the terminal ileum. (B) By magnifying endoscopy, hookworm is seen hooking its head into the intestinal mucosa and engulfing blood. (C) Characteristic hookworm mouthparts. (Courtesy of reference 16, with permission.) doi:10.1128/9781555819002.ch29.f6

sion and pain, diarrhea, and occult blood in the feces, accompanied by peripheral eosinophilia (Fig. 29.6). Whether *A. ceylanicum* is capable of producing both classical hookworm disease and evoking morbidity through an uncontrolled allergic response in some individuals remains unknown.

Halicephalobus gingivalis

The genus *Halicephalobus* consists of eight species of free-living nematodes. Only one species (*H. gingivalis*; formerly *Micronema deletrix* or *Halicephalobus deletrix*) infects vertebrates (18). Human infection is extremely rare, and only four cases have been reported in the literature. These nematodes tend to exhibit neurotropism, but their life cycle, mode of infection, and risk factors are poorly understood. Neurohelminthiases are not generally recognized in the United States, and when they do occur, the diagnosis is often missed. One report concerns a case of meningoencephalomyelitis caused by a *Halicephalobus* sp., in which the patient had a rapidly deteriorating clinical course. Although eosinophils were seen in the cerebrospinal fluid, the case did not raise any clinical suspicion of neurohelminthiases (19).

These roundworms are free-living and are normally found in soil, manure, or decaying humus. *H. gingivalis* is reported to have caused a fatal meningoencephalomyelitis in a 5-year-old boy who accidentally fell into a manure spreader and passed through the mechanism. He sustained multiple severe wounds and fractures, all of which had been heavily contaminated with manure. Although his lesions appeared to heal satisfactorily, 18 days after the injury he became lethargic. Lumbar puncture revealed over 300 cells per ml and equal numbers of lymphocytes and macrophages. The child died of meningoencephalitis 24 days after the accident (20). The autopsy revealed extensive inflammatory foci in the brain, each of which contained various developmental stages of the worms (Fig. 29.7) (21).

Another fatal case of human meningoencephalitis caused by *M. deletrix*/*H. gingivalis* was reported. The patient had severe granulomatous meningoencephalitis with many foci of hemorrhage and encephalomalacia. Many adult female and larval worms were found in the brain. The organisms probably live in decaying organic material but occasionally parasitize the kidneys, nose, or central nervous system of the horse. In its parasitic form, the nematode reproduces parthenogenetically (22).

Oesophagostomum spp.

The roundworms of *Oesophagostomum* spp. infect the intestine or abdominal cavity of humans or animals, normally ruminants, primates, or swine. The disease is most common in the tropics or subtropics, with approximately 60 human cases being reported, primarily from Africa. Several species of *Oesophagostomum* have been implicated in human disease; however, accurate species identification is very difficult if one cannot examine an intact whole worm. The life cycle is seen in Fig. 29.8. Usually, the worms are unable to complete their life cycle in humans; however, there have been instances in which stunted adult worms have been recovered. In northern Togo and Ghana, this infection is found in humans, with the recovery of both adult males and females passed in the stool after therapy (Fig. 29.9 to 29.12) (23). The parasite is *Oesophagostomum bifurcum*, which apparently does not require an animal reservoir. The eggs passed in the stool closely resemble

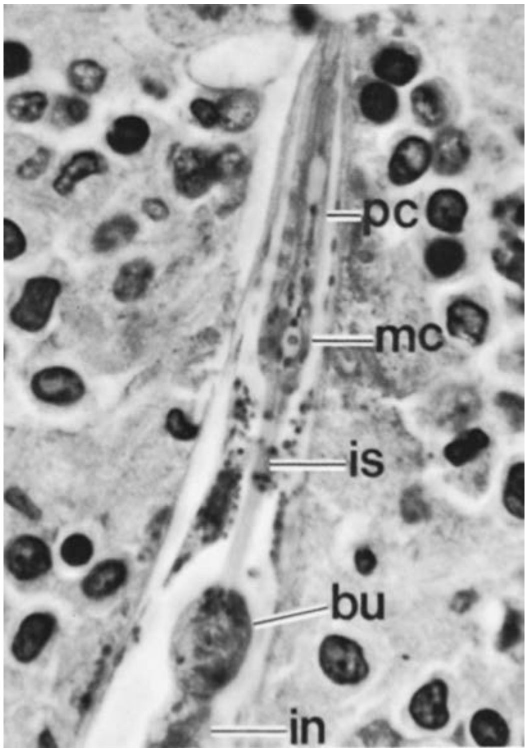


Figure 29.7 Longitudinal section through the rhabditiform esophagus in the anterior end of a mature female *Micronema deletrix* (renamed *Halicephalobus gingivalis*) worm in the brain. The procorpus (pc), metacarpus (mc), isthmus (is), bulb (bu), and intestine (in) are indicated (×1,040). (Armed Forces Institute of Pathology photograph; published in *Can J Neurol Sci* **2**:125, 1975.) doi:10.1128/9781555819002.ch29.f7

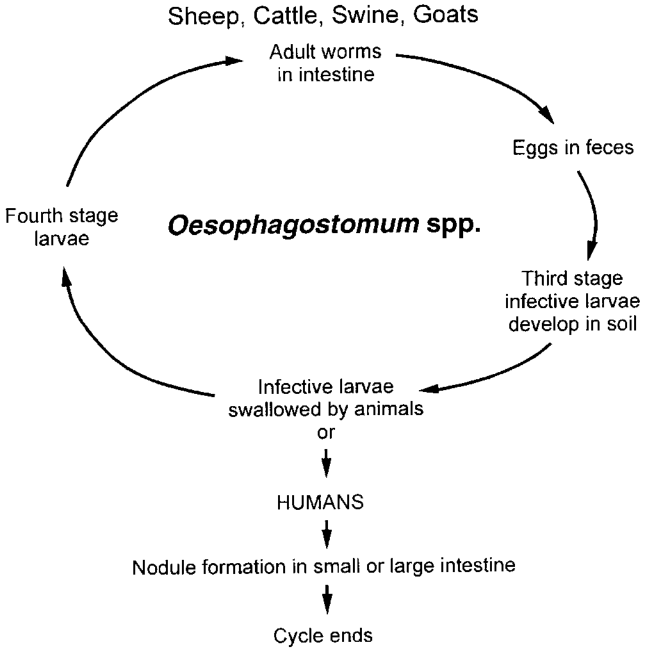

Sheep, Cattle, Swine, Goats
Adult worms in intestine
Eggs in feces
Third stage infective larvae develop in soil
Infective larvae swallowed by animals or
HUMANS
Nodule formation in small or large intestine
Cycle ends
Fourth stage larvae
Oesophagostomum spp.

Figure 29.8 Life cycle of *Oesophagostomum* spp. doi:10.1128/9781555819002.ch29.f8

those of hookworm (23), and both infections are found in this population (Fig. 29.7).

The infection can develop in humans at any age. Most of the worms recovered from humans are immature. Symptoms include abdominal pain, with localized tenderness (usually right lower quadrant). There may be gradual onset of pain, with a low-grade fever and no diarrhea, vomiting, or anorexia (24). Careful examination of the patient may reveal a mass in the abdominal cavity, adhering to the abdominal wall (Fig. 29.11) (25). Although these nodular mass lesions usually do not cause intestinal obstruction, other complications, such as hernia, intussusception, or worm penetration of the bowel wall, may occur (26). The mass is often attached to the bowel wall of the ileum or colon (27). Nodules usually measure 4 to 6 cm. Often carcinoma, tuberculosis, or Crohn's disease may be suspected. In one patient, the worm produced a cutaneous lesion, the first report of a lesion outside of the gastrointestinal tract and without evidence of bowel infection (28).

If oesophagostomiasis is suspected, confirmation can be made by immediate examination of the nodule, either by nodule dissection (Fig. 29.12) or by examination of aspirate material. Definitive diagnosis depends on identifying the whole worm or serial sections of it. At present, surgical resection of the involved tissue or removal of the worm from the nodules is the only means of treatment. However, both pyrantel pamoate and albendazole have been effective in removing adult worms from the intestine (23). It is very likely that a single dose of albendazole will cure an *O. bifurcum* infection (29).

Oesophagostomum spp., as well as hookworm infections, are hyperendemic among the population in northern Togo and Ghana (30, 31). In northern Togo, all the villages examined contained residents who were infected with hookworm, with a patchy distribution of *Oesophagostomum* spp. Women were infected with *O. bifurcum* more often than men were, while infections with hookworm were the opposite. The prevalence and intensity of infection with both parasites were clearly age dependent. It is estimated that more than 100,000 people in Togo are infected with *O. bifurcum* and more than 230,000 are infected with hookworm.

Using a panel of 155 well-defined fecal and DNA samples, a new PCR assay achieved a sensitivity of 94.6% and a specificity of 100% in the identification of *O. bifurcum*. This PCR assay will be useful for the diagnosis of *O. bifurcum* infection, as well as being a molecular biology tool to help clarify the epidemiology of human disease with this parasite (32). When using routine diagnostic methods to estimate the intensity of infection, it is sufficient to make repeated coprocultures from only a single stool

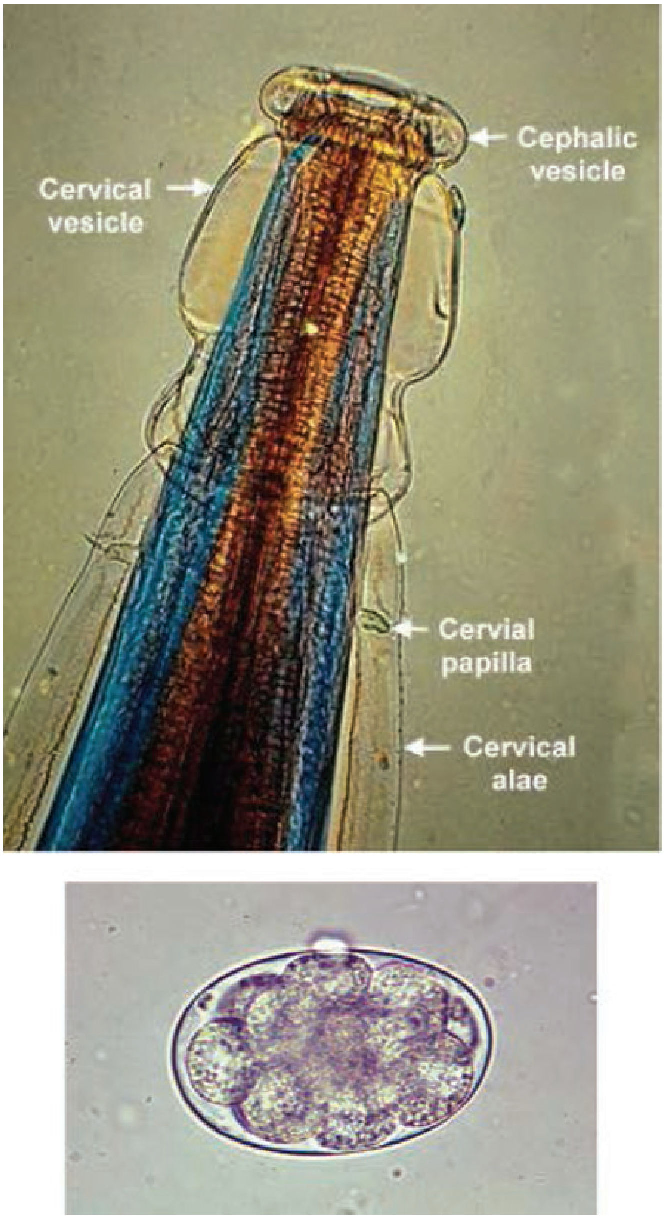


Figure 29.9 (Upper) Anterior end of an adult *Oesophagostomum bifurcum* worm recovered from human feces. Note the cephalic groove, characteristic of the genus (×115). (Reprinted from Merial U.S./animal health company, cited as original source from http://cal.vet.upenn.edu/projects/merial/Strongls/strong_6.htm.) (Lower) *Oesophagostomum* egg; this egg cannot be differentiated from that of hookworm (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch29.f9

sample; collection of multiple samples on subsequent days does not give better estimates of the individual infection status (32). It is also important to remember that coproculture can also yield larvae of *Oesophagostomum* and *Ternidens* species, as well as the more common hookworm and *Strongyloides stercoralis* (33).

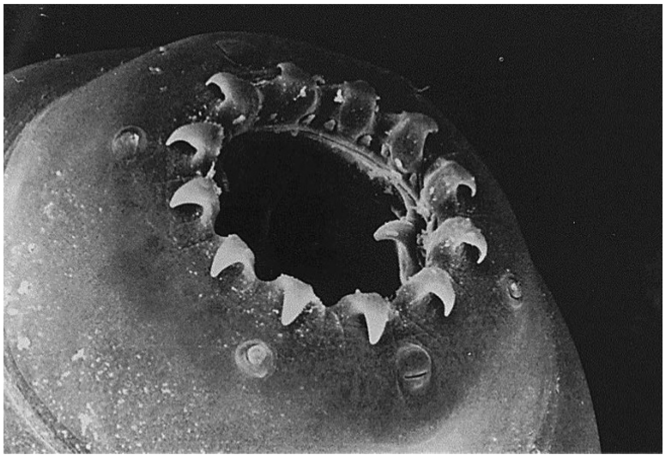
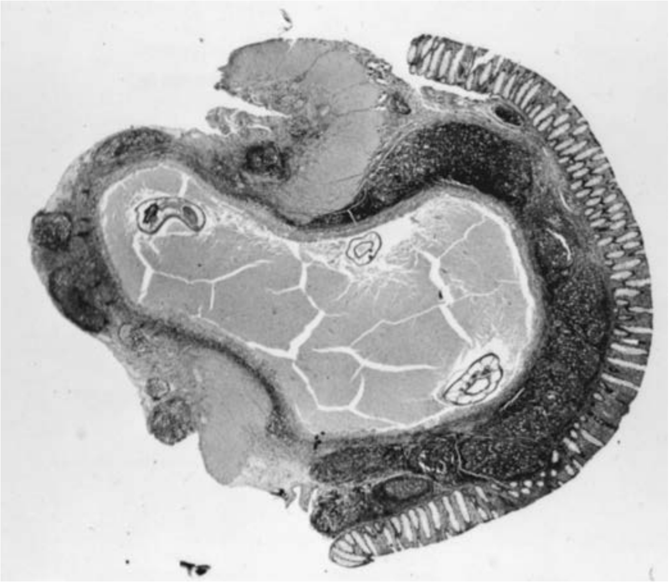


Figure 29.10 Scanning electron micrograph of the cephalic end of a female *Oesophagostomum bifurcum* worm recovered from human feces. Note the inner and outer leaf crowns with 12 and 24 (sometimes 10 and 20) lips, respectively, and the papillae (×630). (Photograph by the Department of Electron Microscopy, Medical Faculty, Leiden University; reprinted from reference 15 with permission.) doi:10.1128/9781555819002.ch29.f10

Eustrongylides spp.

The larvae of *Eustrongylides* nematodes are found in raw fish and in amphibians and reptiles. Although not all fish hosts are known, it was assumed that, in one case, the

Figure 29.11 Nodular lesion of oesophagostomiasis seen as a paraumbilical mass in an otherwise healthy 5-year-old girl in Nigeria. At laparotomy, the lesion was found to have arisen in the ascending colon and was adherent to the muscles and fascia of the anterior abdominal wall (×11). (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch29.f11



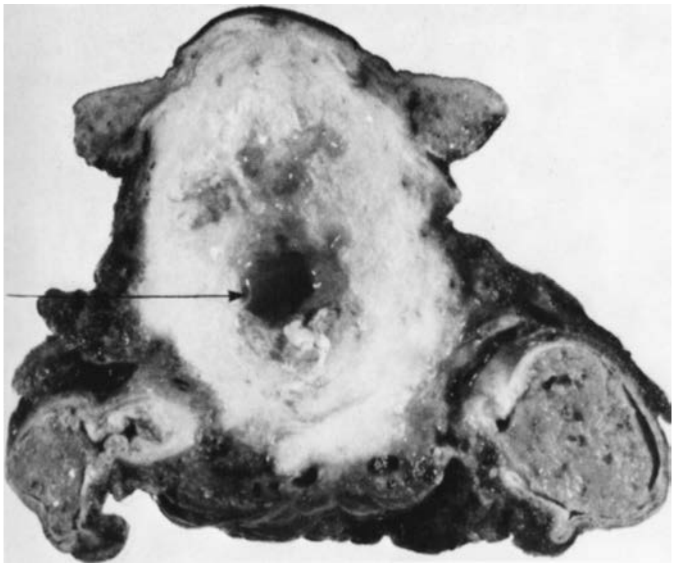


Figure 29.12 Surgically resected nodule of oesophagostomiasis. Note the worm protruding into the cavity. Specific identification of these worms is difficult, because intact specimens are rarely obtained. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch29.f12

patient most probably acquired the infection by eating raw fish (sashimi and sushi) approximately once a month (Fig. 29.13). He usually ingested the fish in restaurants; however, the day prior to admission, he had eaten raw fish of an unknown species from a neighborhood fish market. He presented with a history of pain in the right lower quadrant. On the basis of the symptoms, the patient underwent an appendectomy, at which time the appendix and distal ileum were found to be normal. However, prior to closure of the incision, a pinkish red worm was seen crawling onto the surgical drapes from the abdominal cavity. The patient's recovery was unremarkable. On the basis of larval morphology, the worm was determined to be in the *Eustrongylides* group, which includes parasites that are commonly found in fish-eating birds (34). A second patient also presented with symptoms simulating acute appendicitis. The patient had consumed two live minnows obtained from Big Timber Creek, Bellmawr, NJ. The parasite *Eustrongylides ignotus* was recovered surgically from this patient (35). Human infection with *Eustrongylides* spp. is certainly uncommon, but with the increased consumption of raw or improperly cooked fish, additional cases may be seen. The majority of cases have been related to anisakine larvae such as those of *Anisakis*, *Phocanema*, *Contracaecum*, and *Pseudoterranova* spp., which are indigenous to marine fish found off both coasts of the United States as well as in Japan and Europe.

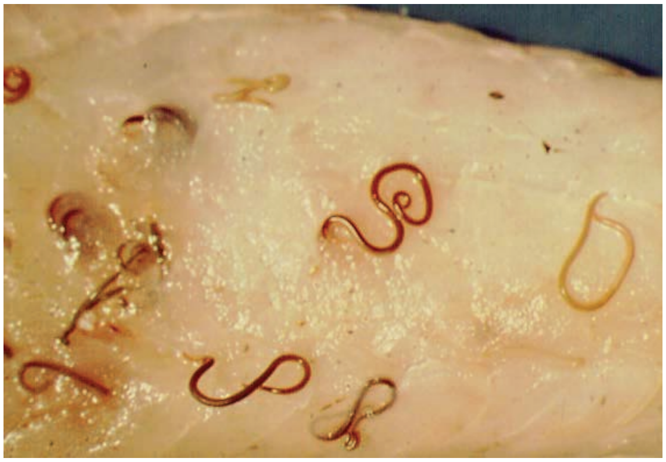


Figure 29.13 *Eustrongylides* nematode larvae in raw fish (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). doi:10.1128/9781555819002.ch29.f13

Two large, living worms were collected as they emerged from the lower limb of each of two persons in South Sudan. The worms measured 7 and 8 cm in length and were identified as fourth-stage larvae of *Eustrongylides*. This is the first report of such worms emerging from the skin; all five previous reports of human infection involved surgical removal of worms from the peritoneal cavity (36).

Mermis nigrescens

Mermis nigrescens nematodes are found in the soil and have a unique life cycle. After mating and egg maturation, the females leave the soil and deposit eggs on the aboveground portions of plants. The normal hosts are grasshoppers, which become infected by ingesting eggs while feeding on vegetation. After completing their development in the grasshoppers, the worms emerge and enter the soil, where mating occurs (Fig. 29.14). One specimen that was removed from the mouth of a 1-month-old infant was identified as a gravid female *M. nigrescens* (Fig. 29.15). On the basis of the parasite life cycle, it was assumed that the female was ovipositing on vegetation and was carried, perhaps on the fur of a dog or cat present in the house, to the vicinity of the child. The parasite was possibly attracted to the moisture of the mouth (37).

Dioctophyma renale

The large (14 to 20 cm long by 46 mm wide) roundworm *Dioctophyma renale* is widely reported to have been isolated from dogs. Infection in humans is acquired from the ingestion of raw freshwater fish and frogs. The adult

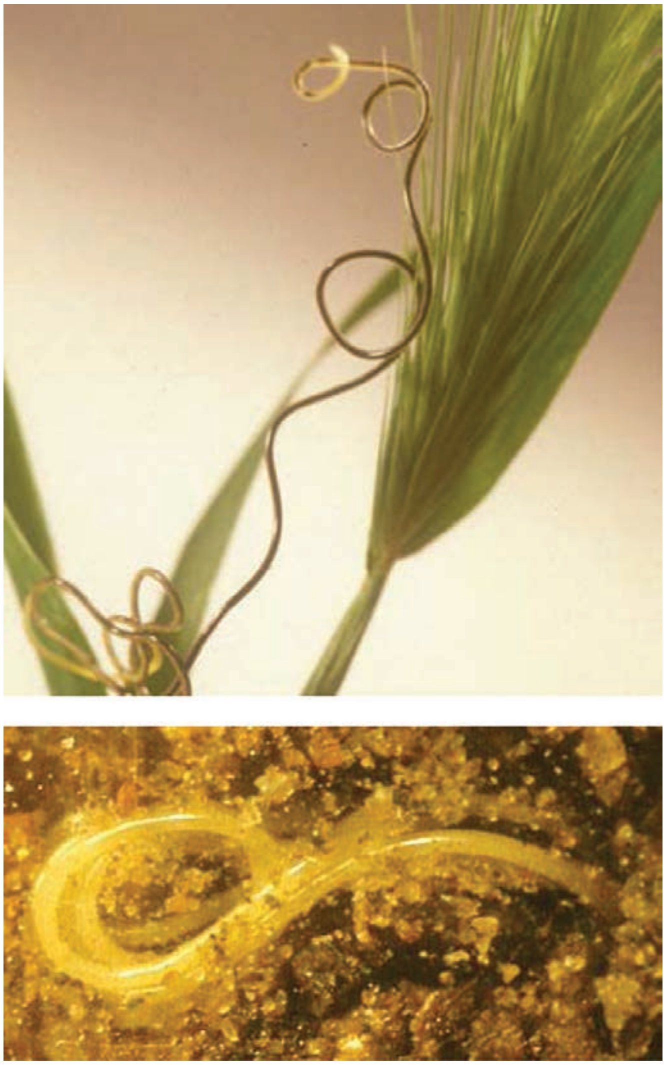


Figure 29.14 (Upper) Adult female grasshopper nematode, *Mermis nigrescens*, climbing vegetation in preparation for depositing eggs. (Lower) Juvenile grasshopper nematode, *M. nigrescens*, that has descended into the soil after exiting a grasshopper (courtesy of University of Florida, photograph by John Capinera: http://entnemdept.ufl.edu/creatures/beneficial/misc/mermis_nigrescens.htm). doi:10.1128/9781555819002.ch29.f14

worms usually live in the pelvis of the right kidney or in body cavities. The kidney parenchyma is gradually destroyed, leaving only the capsule. In all but 1 of the approximately 13 reported human cases, the kidney was involved (38). Infections were discovered at autopsy, by detection of worms migrating from the urethra, by detection of a worm discharged through the skin over an abscessed kidney (25), and by recovery of eggs in the urine (25) (Fig. 29.16).

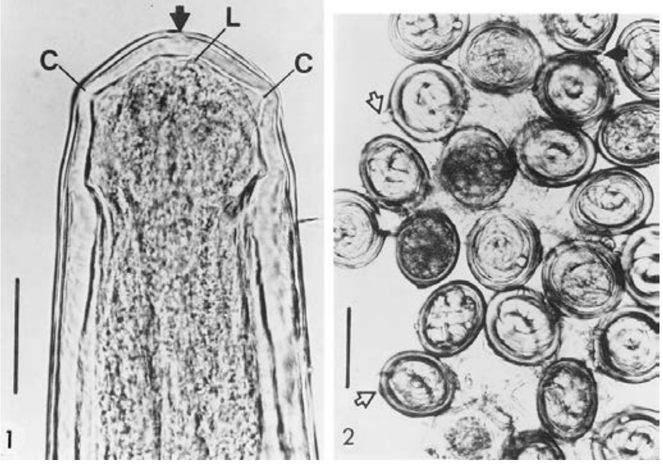
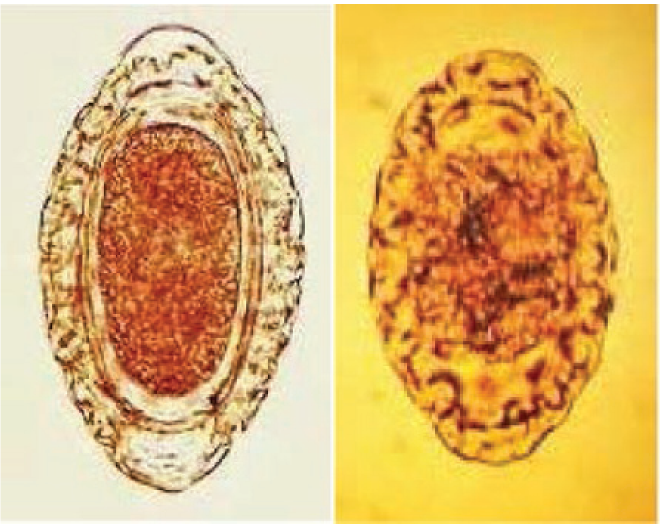


Figure 29.15 (1) Head of *Mermis nigrescens* removed from the mouth of an infant. Note the mouth opening (arrow), lip papilla (L), and cephalic papillae (C). Bar, 80 µm. (2) Eggs of *M. nigrescens* inside the uterus of the specimen. Note the thick, double-walled shell surrounding the fully developed embryos. Arrows point to poles containing collapsed byssi. Bar, 55 µm. (Reprinted from reference 37 with permission.)
doi:10.1128/9781555819002.ch29.f15

A 50-year-old Chinese man was found to have a retroperitoneal mass in the right upper quadrant of the abdomen by ultrasonography and computed tomography. At surgery, a hemorrhagic cyst was seen at the upper pole of the right kidney adjacent to the adrenal gland. Histologic examination revealed that the granulomatous cyst wall was filled with eggs and cross sections of parasites that were subsequently identified as *D. renale*. The eggs had a

Figure 29.16 *Dioctophyma renale* eggs. These eggs measures 60 to 80 µm by 39 to 46 µm and have a thick shell that appears wrinkled; the ends of the egg are usually lighter in color (see egg on the left). doi:10.1128/9781555819002.ch29.f16



birefringent striated double wall (Fig. 29.17). The right kidney was intact, and no eggs were seen in the urine (39).

Liesegang rings (LR) are acellular, laminated structures that may be deposited within and around cysts and inflamed or necrotic tissue. Previous reports have shown that the kidney and breast are organs commonly affected with LR (Fig. 29.18 and 29.19). LRs in tissue are periodic precipitation zones from supersaturated solutions in colloidal systems. They are formed by a process involving interplay of diffusion, nucleation, flocculation or precipitation, and supersaturation. In a description of LRs from the lesions of 29 patients, the LRs formed in cysts or in fibrotic, inflamed, or necrotic tissue. Special stains and energy-dispersive radiographic analysis or scanning electron microscopy revealed that some LRs contained calcium, iron (hemosiderin), silicon, and sulfur. Some pathologists have mistaken LRs for eggs, larvae, or adults of *D. renale*. It is important to remember that these LRs can be mistaken for parasites and have been found in tissues and from renal cyst fluid (40–42). LRs are most commonly seen in fine-needle aspiration cytology preparations, but they can also be seen in histological tissue sections.

Ternidens deminutus

The nematode *Ternidens deminutus* is found as a parasite of the large intestine of several simian hosts and is occasionally found in humans. Human infection occurs when food contaminated with infective filariform larvae is ingested. The larvae molt in the intestinal wall and become adults. They pass eggs in feces, and eggs in contaminated soil hatch and become rhabditiform larvae and then infective filariform larvae. The worms inhabit the wall of the large intestine and may produce ulcers or cystic nodules (43). In one case, a *T. deminutus* infection was reported in a 33-year-old Thai female who was admitted to the hospital because of abdominal pain and a right lower quadrant mass. Exploratory laparotomy revealed an omental mass with attached terminal ileum. Resection of the mass together with the terminal ileum and the right-sided colon was performed. Histologically, the omental mass was an abscess containing an immature male *T. deminutus* (44).

Figure 29.17 *Dioctophyma renale* in human tissue; cross sections of larvae of *D. renale* in a subcutaneous nodule (courtesy of the CDC Public Health Image Library).
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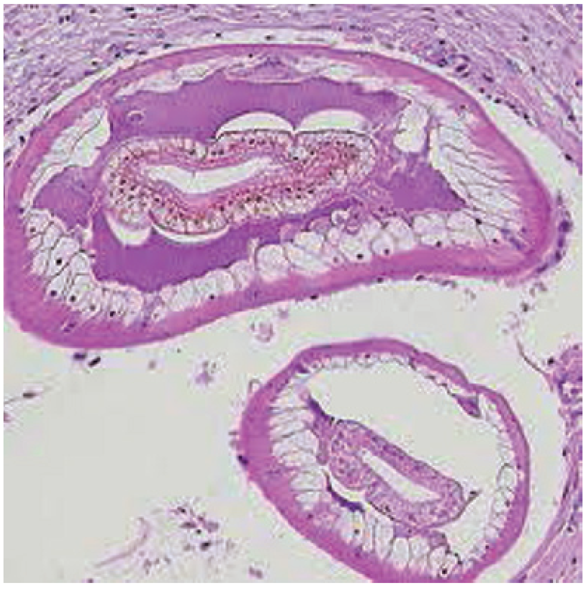

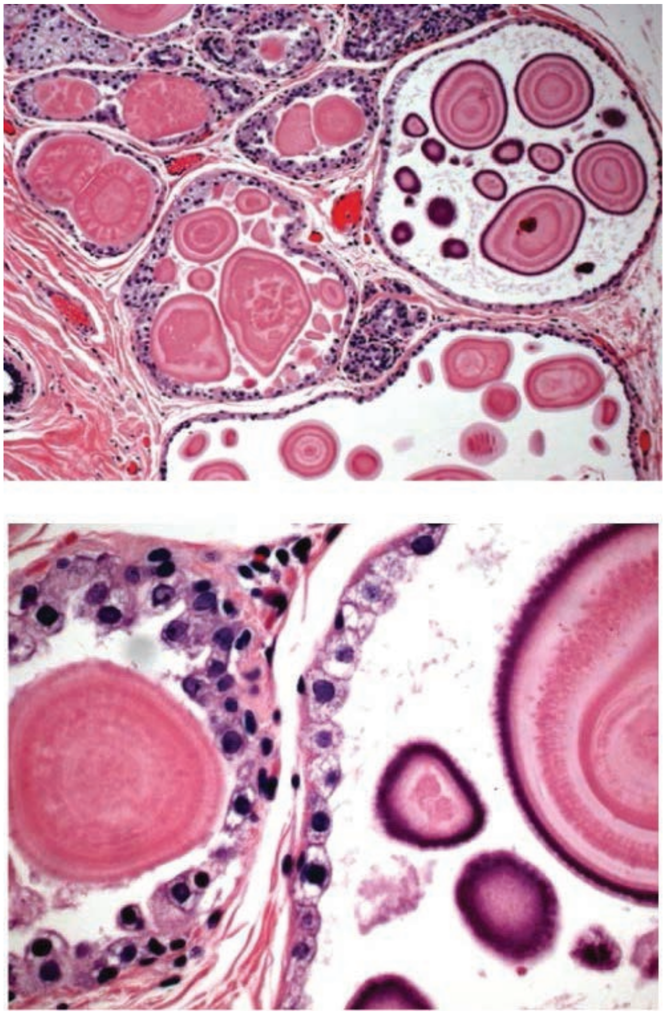

Figure 29.18 Liesegang rings. (Upper) Liesegang-like rings in slightly dilated breast ducts associated with lactational change (hematoxylin and eosin; original magnification, ×10). (Lower) Higher magnification of Liesegang-like rings and lactational change (hematoxylin and eosin; original magnification, ×40). (Courtesy of reference 42; National Institutes of Health.)
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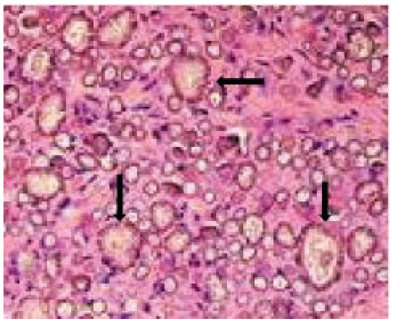

Figure 29.19 Liesegang rings in the wall of a hemorrhagic renal cyst (arrows) (hematoxylin and eosin, ×200) (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch29.f19

The geographic range includes Africa, India, and Indonesia; human infections have been reported in Southeast and South Africa. Although it is apparently fairly common in natives of Zimbabwe and has been found in large numbers in certain specific communities, it is not found in Europeans (43). Morphologically, *T. deminutus* resembles a hookworm; however, the anterior buccal capsule is guarded by a double crown of 22 stout bristles. The oval eggs closely resemble those of human hookworms but are larger (84 by 51 µm) (Fig. 29.20). Third-stage larvae develop in soil or Harada-Mori cultures in 8 to 10 days; however, the complete life cycle is unknown. Newly identified genetic markers provide a foundation for an improved PCR-based diagnosis of these infections (45). Both thiabendazole and pyrantel pamoate produce cure rates over 90%.

Figure 29.20 *Ternidens deminutus* egg. This egg measures 85 by 50 µm and closely resembles the egg of hookworm and *Oesophagostomum*; however, it is larger. (Left) Drawing; (right) iodine preparation direct mount.
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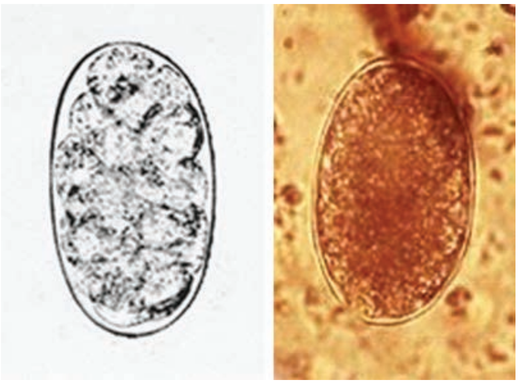


Mammomonogamus laryngeus (*Syngamus laryngeus*)

Mammomonogamus laryngeus is commonly found in the upper respiratory tract of some mammals, including cattle and felines. There have been approximately 100 cases of human infection, with almost all cases being reported from the Caribbean islands; more than half of the cases occurred in Martinique or in France in people who had traveled to Martinique (44, 46). Other reports have mentioned Puerto Rico, Dominica, St. Lucia, Trinidad, Guyana, Brazil, and the Philippines. Although third-stage larvae are probably infective for the vertebrate host, little is known about the life cycle.

The worm is red to reddish brown because of the ingestion of blood (46). The male and female worms are permanently joined *in copula* and present a Y shape. The short arm of the Y is the male worm, and the longer arm and base of the Y is the female worm (Fig. 29.21.) Both worms have a thick-walled buccal capsule which is armed in the inner base with eight small teeth. Male worms measure 3.0 to 6.3 mm long and 360 to 380 µm wide. Female worms measure 8.7 to 23.5 mm long and 550 to 570 µm wide. The eggs are oval, have a thick cortical shell, and measure 78 to 95 µm long by 42 to 54 µm wide (Fig. 29.22).

The worms in the trachea are often associated with a severe, nonproductive cough; the cough is often accompanied by hemoptysis and occasionally asthma, possibly because of obstruction of the bronchi or bronchioles. Eosinophilia may or may not be present. Diagnosis can be performed by examination either of worms or eggs coughed up in the sputum or of eggs that may be swallowed and recovered in the stool. Direct visual examination of the throat and/or endoscopy may also reveal the presence of worms; worms can be removed by using forceps or surgical techniques. There have been no controlled studies of specific therapy of human infections with *M. laryngeus*. In one case reported, an Italian tourist returning from the Caribbean presented with chronic cough and episodes of hemoptysis. The patient had many diagnostic procedures and was repeatedly, yet unsuccessfully, treated for asthma, bronchitis, and gastroesophageal reflux disease before the correct diagnosis was reached. During a fiber optic bronchoscopy, a Y-shaped red object was extracted and identified as a pair of *Mammomonogamus laryngeus* worms. After this procedure the patient improved, although a dry cough persisted and two other minor episodes of hemoptysis occurred. The patient was treated with ivermectin and thiabendazole and recovered after 3 months; the second fiber optic bronchoscopy was negative (47). In another case, a pair of *M. laryngeus* roundworms *in copula* was recovered from the duodenum of a 72-year-old male Thai

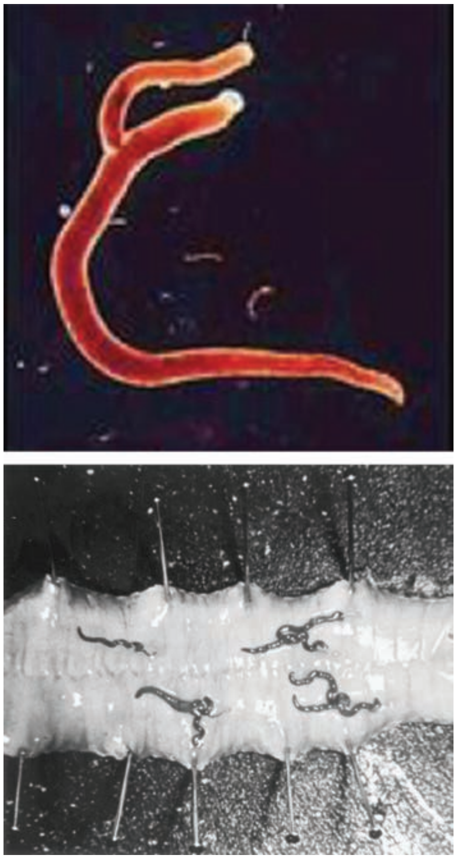


Figure 29.21 *Mammomonogamus laryngeus* (*Syngamus trachea*). (Upper) “Y” configuration. (Lower) Worms in the trachea of a turkey; note the typical “Y” configuration of the adult male and female worms. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch29.f21

patient from Kanchanaburi Province, Thailand. Eggs were also found in the stool. Apparently, this is the first case of infection originating in Thailand, since the previous two reports attributed the infection as originating in Malaysia. The occurrence of adult worms in the duodenum is unusual and differs from previous findings in the larynx, posterior pharynx, tracheal wall, and bronchi (48).

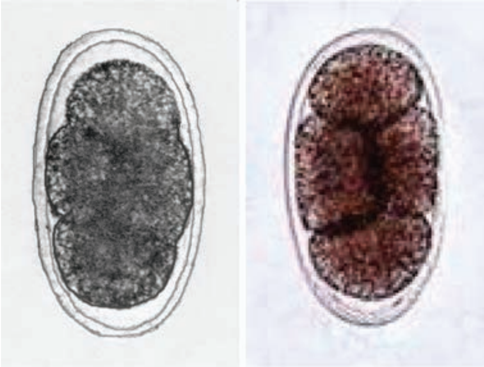


Figure 29.22 *Mammomonogamus laryngeus* eggs. This egg measures 78 to 95 µm by 42 to 54 µm. (Left) drawing; (right) iodine preparation direct mount.
doi:10.1128/9781555819002.ch29.f22

M. laryngeus is a common veterinary parasite, and the adult worms are normally found in the laryngotracheal region of ruminants. The eggs are coughed up and swallowed and can be found in the sputum or stool. Once the eggs are outside the host, they undergo development until the larva becomes infective; the larva can remain in the egg or be released into the environment. Ruminants become infected through the ingestion of food or water contaminated with infective larvae. Carrier hosts such as earthworms, snails, or arthropods may also play a role in infection. Apparently, the larvae are released in the digestive tract, cross the intestinal wall to the mesenteric veins, and migrate to the tracheolaryngeal area, where the adult worms mate and begin reproduction (46).

Ascaris suum

Human infection with adult *Ascaris suum* is occasionally documented, based primarily on very specific worm morphology and relevant history with potential exposure to pigs. Generally, *Ascaris lumbricoides* and *A. suum* are morphologically so similar that individual worms usually cannot be identified as one or the other.

Of much greater concern are the human infections with *A. suum* in which the larvae cause visceral larva migrans (VLM) and invade the liver, lungs, and the intestine but do not mature to adult worms (49). In some cases, encephalopathy can also be associated with VLM caused by *A. suum*. In one case, the patient suffered from drowsiness, quadriparesis, eosinophilia, and elevated IgE levels in serum. Magnetic resonance imaging revealed multiple cerebral cortical and white matter lesions, and serologic test results indicated recent infection with *A. suum*.

Patients with VLM have respiratory symptoms; although eosinophilia is marked, no eggs or larvae have been found in stool specimens. Multiple small mass lesions can be seen on magnetic resonance imaging; they all disappear after anthelmintic therapy. Although liver tissue biopsy specimens showed marked infiltrations by eosinophils in the portal tracts and hepatic sinusoids, no larvae or eggs were seen (50). Hepatic nodules have been located mainly in periportal or subcapsular regions, which may represent periportal eosinophilic granuloma (51).

The number of infective eggs required to initiate an infection is apparently quite small; massive infections with *A. suum* can cause severe disease (38), However, the actual significance of human infection with this nematode is not known. Among people who visit or live on farms where pigs are raised, *A. suum* may be a significant cause of illness.

Recent molecular studies provide evidence that *A. lumbricoides* and *A. suum* represent a single species (52). Other results suggest the existence of homogenizing gene flow between these two taxa, which appear to be variants of a single polytypic species (53).

Gongylonema pulchrum

The thin nematode *Gongylonema pulchrum* is a common parasite of ruminants and has been diagnosed in bears, hedgehogs, monkeys, pigs, and occasionally humans. Human cases have been reported from Europe, Morocco, the former USSR, China, New Zealand, Sri Lanka, and the United States. Human infection is acquired by accidental ingestion of infected insects. Pathology in human cases is due to maturing and adult worms migrating through the mucosa and submucosa of the buccal cavity and the esophageal mucosa. Worms have been recovered from the lips, gums, hard and soft palate, tonsils, and angle of the jaw. In some cases, blood is present. In a case report from the United States, a 41-year-old woman living in New York City presented with a history of a sensation of a "moving organism" in her mouth for more than a year (54). On two occasions she removed worms from her mouth, once from the lip and once from the gum. The patient also reported a history of reflux-like symptoms that may have been associated with esophageal involvement. The worm extracted from the lip was identified as a mature female *Gongylonema* worm, probably *G. pulchrum*. The anterior was very thin and threadlike, with a diameter of 0.5 mm; it was covered with typical bosses (scutes) arranged in longitudinal rows (Fig. 29.23). The posterior end of the worm was considerably wider. The eggs measured 50 by 28 µm.

In another case, a 48-year-old man complained of itching in various parts of the buccal mucosa over the preceding weeks. The patient lived in a rural region in southern Alsace and had never visited Africa. He worked overseeing the unloading of flour at the port of Basel. The initial clinical examination was negative. However, the patient photographed a filament previously present in his buccal mucosa, and he extracted a worm. The latter was identified at the Strasbourg Institute of Parasitology as *G. pulchrum* (55).

The 13th reported case of human infection with *Gongylonema* spp. in the United States, and the first to be confirmed as *G. pulchrum*, was reported in 2013.The parasite described was isolated from the oral cavity of a resident of Williamsburg, VA. The identity of the parasite was verified through morphological and genetic approaches and provided the first genetic confirmation of a *Gongylonema* sp. in humans (56).

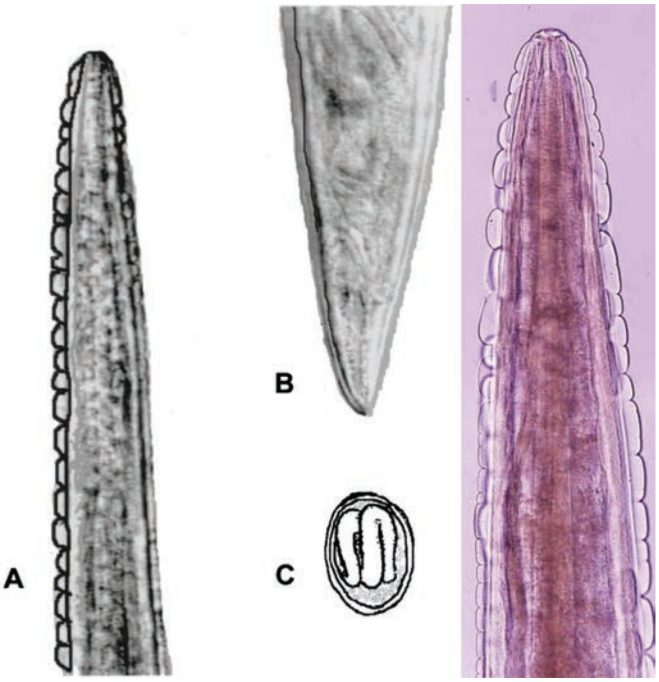


Figure 29.23 *Gongylonema pulchrum*. (A) Anterior end of the worm (adapted from Ward HB, *J Parasitol*, 1916). (B) Posterior end of the worm (adapted from reference 4). (C) Egg (adapted from reference 54). (Far right) Anterior end of the worm. doi:10.1128/9781555819002.ch29.f23

Haycocknema perplexum

A new genus of nematode, *Haycocknema perplexum*, causes polymyositis in humans. Adult male and female nematodes and larvae were recovered from myofibers after biopsy of the right vastus lateralis muscle. This infection in a man in Tasmania, Australia, was associated with a polymyositis (57). Two other potential cases have been reported, one of which was also in a patient from Tasmania (58). A muspiceoid nematode causing polymyositis was involved in these cases. These minute adult worms are very slender (<35 µm) and measure approximately 300 µm (male) and 425 µm (female) in length. The adult worms are very small and may be mistaken for larvae. While treatment with prednisone produced no improvement, subsequent treatment with albendazole led to immediate improvement.

Another case was reported as the sixth case from Australia. A 50-year-old man from Tasmania had a 2-year history of progressive weakness, weight loss of 10 kg, and dysphagia. The muscle biopsy was initially interpreted as polymyositis with eosinophils. He deteriorated after several months of treatment with prednisolone and methotrexate, and subsequent review of the muscle biopsy showed intramyofiber parasites of *H. perplexum*. After 3 months of treatment with albendazole therapy, he recovered, and his creatine kinase decreased to 470 U/liter. This uniquely Australian parasite can mimic polymyositis and leads to significant irreversible morbidity and even mortality, if diagnosed late or after corticosteroids. Diagnosis is based on histopathology of muscle biopsy tissue (59). Muscle weakness, eosinophilia, and biopsy results have been consistent with an active polymyositis.

Cestodes

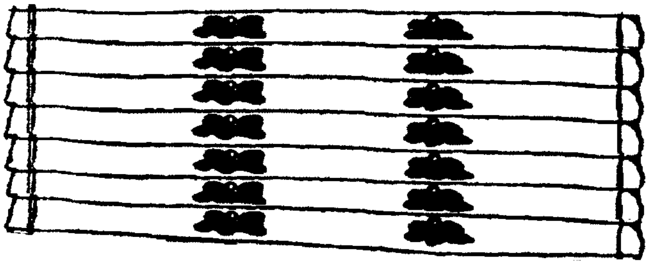
Although *Echinococcus oligarthrus* and *Echinococcus vogeli* were covered in this chapter in previous editions of this book, they have been moved to chapter 17. A new species, *Echinococcus shiquicus*, has been identified from China. Although no human infections have yet been identified, the possibility of infection certainly exists.

Diplogonoporus spp.

Diplogonoporus grandis and *Diplogonoporus balaenopterae* have been identified as tapeworm parasites of humans. This tapeworm is normally found in whales but has been isolated from a number of Japanese patients (38). The adult worm measures 1.4 to 5.9 m long and has gravid proglottids that are very wide (15 to 25 mm) and very short (0.54 mm). There are two sets of genital organs in each proglottid (Fig. 29.24). The eggs are operculated, their morphology is very similar to that of eggs of *Diphyllobothrium latum*, and they measure 63 to 68 μm by 50 μm (Fig. 29.25).

In one case reported from Spain, a 58-year-old man who consumed raw fish passed a short chain of gravid proglottids. After therapy with 50 mg of paromomycin sulfate per kg divided into three doses, all given on the same day, the worm was finally evacuated. The man had not been out of Zaragoza Province in Spain for approximately 20 years, and the infection was probably associated with the importation of fish into Spain. Outside of the Far East, this is the first confirmed case of diplogonoporiasis. The viability of the infective larval stage is evidence of resistance to export/import conditions (60).

Figure 29.24 *Diplogonoporus* tapeworm proglottids. Note the width of the proglottids and their very short length; also note the two genital organs in each proglottid. doi:10.1128/9781555819002.ch29.f24



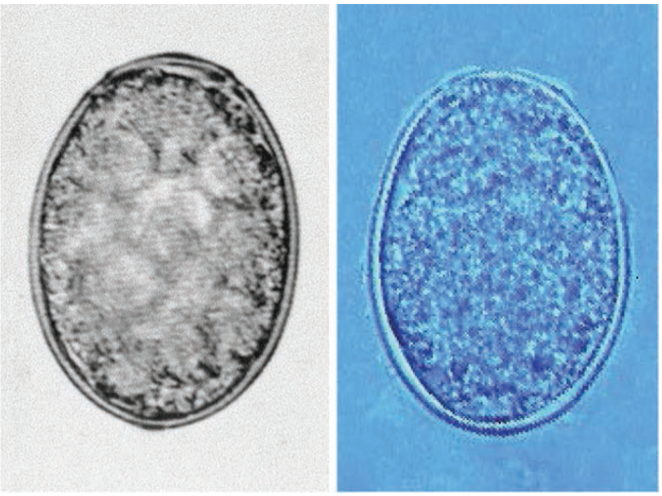


Figure 29.25 *Diplogonoporus* egg. This egg measures 63 to 68 μm by 50 μm; note the resemblance to the *Diphyllobothrium latum* egg. (Left) Drawing; (right) methylene blue preparation direct mount. doi:10.1128/9781555819002.ch29.f25

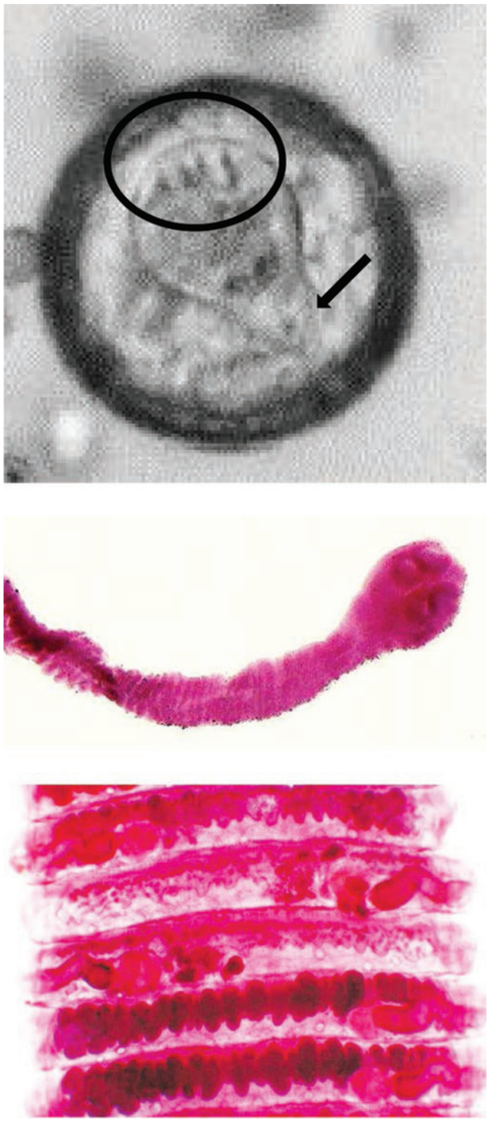
An additional 46 cases were found on the Pacific coast of Central Japan in 1996. The cases were predominantly elderly male patients over 50 years of age. Transmission was probably due to the consumption of raw juvenile Japanese anchovy (*Engraulis japonicus*), which are seasonally caught in the spring months off the Pacific coast. In almost a hundred years after its discovery in 1894, there had been more than 180 cases of human diplogonoporiasis recorded in Japan. The high incidence within a relatively short time frame of this investigation was regarded as unusual (61).

Bertiella studeri

The tapeworm *Bertiella studeri* was originally recovered from an orangutan in Borneo and has been isolated from approximately 30 patients from India, Sri Lanka, Malaysia, Sumatra, Java, Borneo, Vietnam, Singapore, Philippines, East Africa, Yemen, St. Kitts, and British West Indies (62-66). The worm is relatively small, measuring up to 300 mm long and 10 mm wide. The proglottids are shed about 20 at a time; each mature proglottid is approximately 6 mm broad by 0.75 mm long. The eggs have an irregular, oval shape and measure 45 to 46 µm by 49 to 50 µm. There is a delicate middle envelope and an inner shell with a bicornuate protrusion on one side (Fig. 29.26).

Infection is acquired by the accidental ingestion of mites containing the cysticercoid larval stage of the worm. After therapy with niclosamide or other anthelmintics, the worms are evacuated; they may also be passed spontaneously, as seen with other tapeworms. Patients may be asymptomatic or complain of vague intestinal disturbances. Some symptoms include intermittent epigastric pain after meals, accompanied by nausea, diarrhea, and anorexia, with no fever or weight loss (62). The eggs are very characteristic, and identification can be confirmed by finding the eggs or a chain of proglottids in the stool. Most of the confirmed human infections have been found in children. Although both niclosamide (Yomesan) and albendazole have been used for therapy, treatment is not always successful (63).

Figure 29.26 *Bertiella studeri*. (Top) Egg; note the pyriform apparatus (arrow) and hooklets (oval). (Middle) Scolex. (Bottom) Proglottids. (Courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch29.f26



Human infections with *Bertiella mucronata* have also been found; however, fewer than 10 cases have been recorded. One infection has been recorded in a child in Fargo, ND, who may have come in contact with an imported monkey (38). Other cases from Saudi Arabia, India, and China have also been reported (64–66).

Inermicapsifer madagascariensis

The tapeworm *Inermicapsifer madagascariensis* is often seen in humans in Havana, Cuba; in fact, outside of Africa, where it causes a disease of rodents, it may be restricted to humans. Although the life cycle has not been established, an arthropod vector is thought to be involved in human infections, which are most common in children aged 1 to 3 years. Symptoms are vague, but there may be anorexia, weight loss, abdominal pain, or irritability (67). Diagnosis is performed by finding the rice grain-like proglottids; however, these proglottids, eggs, or egg capsules are extremely similar to those of *Raillietina* spp. Specific treatment with praziquantel has been reported to be effective in eliminating the tapeworm (Fig. 29.27) (68).

Raillietina celebensis

The tapeworm *Raillietina celebensis*, common in rats, has also been found in humans, mostly children (Fig. 29.28). The intermediate hosts are various species of ants. Usually, the infection causes no symptoms. The proglottids are small (3 mm or less) and resemble those of *I. madagascariensis*.

The adult worm is approximately 40 cm long; each egg packet contains one to four large eggs measuring 90 by 46 µm. Any symptoms that might be present tend to disappear after treatment with niclosamide.

A number of other species in the genus *Raillietina* have been implicated in human disease, although the number of cases has been small. *Raillietina demerariensis* has been found in approximately 5% of patients in the rural areas near Quito, Ecuador; cases have also been reported from Cuba. The adult worms measure up to 60 cm long by 3 mm wide and have around 5,000 proglottids (38). The individual proglottids resemble rice grains and contain about 200 to 250 egg packets, each measuring about 200 µm in diameter. The oval eggs contain very conspicuous hooklets. *Raillietina asiatica*, *R. garrisoni*, and *R. siriraji* all cause infection in humans, and, again, children rather

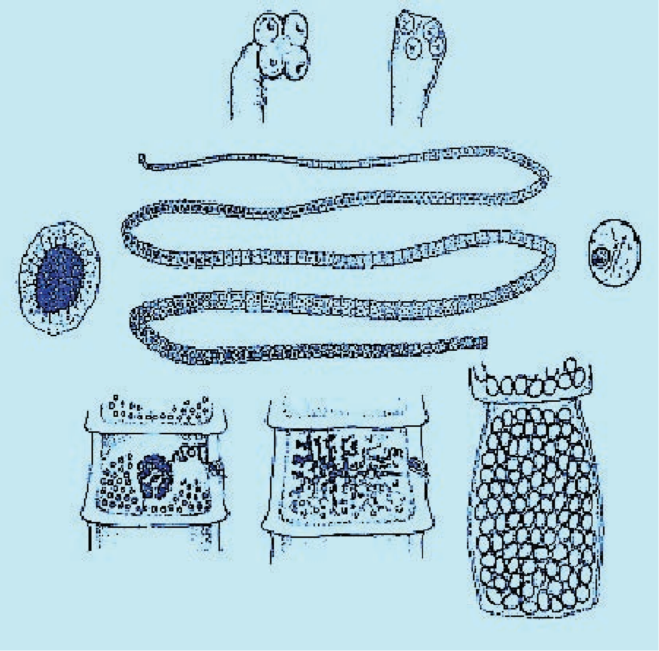


Figure 29.27 *Inermicapsifer madagascariensis*. (Top) Scolex (two views). (Middle, left to right) Egg capsule, strobila, single egg containing a hexacanth larva. (Bottom, left to right) Mature proglottid, young gravid proglottid, gravid proglottid containing egg packets. (Adapted from Kouri P, *J Parasitol* **26:**179–181, 1940; appeared on page 507 of reference 38). doi:10.1128/9781555819002.ch29.f27

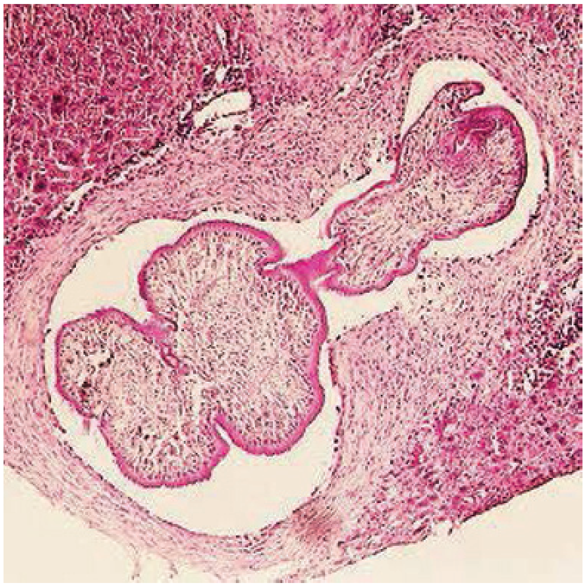


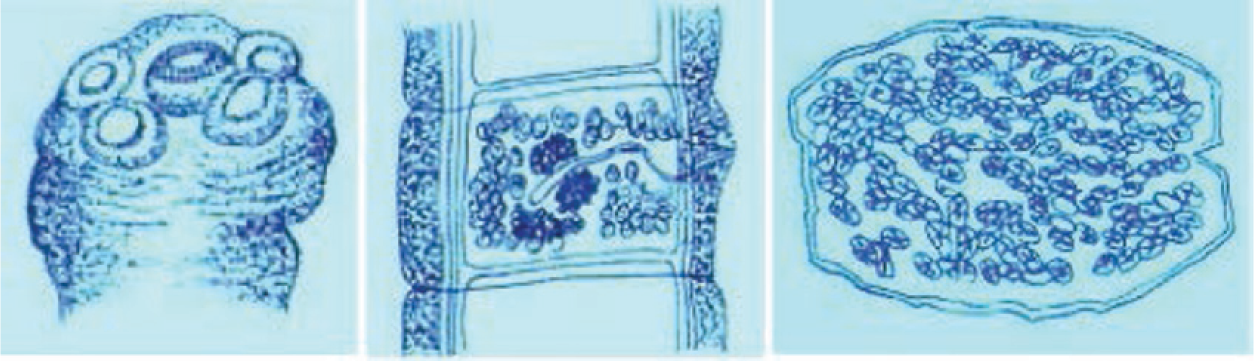
Figure 29.29 *Mesocestoides* spp. Tetrathyridium larva contained in meat from animal that serves as the intermediate host and which is ingested by the human. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch29.f29

than adults are affected. Accidental ingestion of arthropod intermediate hosts is implicated in transmission.

Mesocestoides spp.

Approximately 27 cases of human infection have been reported, probably acquired from ingestion of the larval form (tetrathyridium) in inadequately cooked meat from some animal that serves as the intermediate host (Fig. 29.29). The tapeworm in one case measured 40 cm by 2 mm (Fig. 29.30). The infected child was irritable, had a poor appetite and abdominal colic, and was anemic (69). A second case was reported from Korea (70). The patient, who was a farm worker, complained of abdominal pain and passed a number of proglottids in the stool over a period of several months. The proglottids were described as looking like sesame seeds. Adult worms were recovered after therapy with niclosamide and consisted of 32 complete worms. This is probably the heaviest worm burden reported in a human case. The patient had a history of frequently eating raw viscera of chickens. The seventh

Figure 29.28 *Raillietina celebensis*. (Left) Scolex; (middle) mature proglottid; (right) gravid proglottid containing egg packets. doi:10.1128/9781555819002.ch29.f28



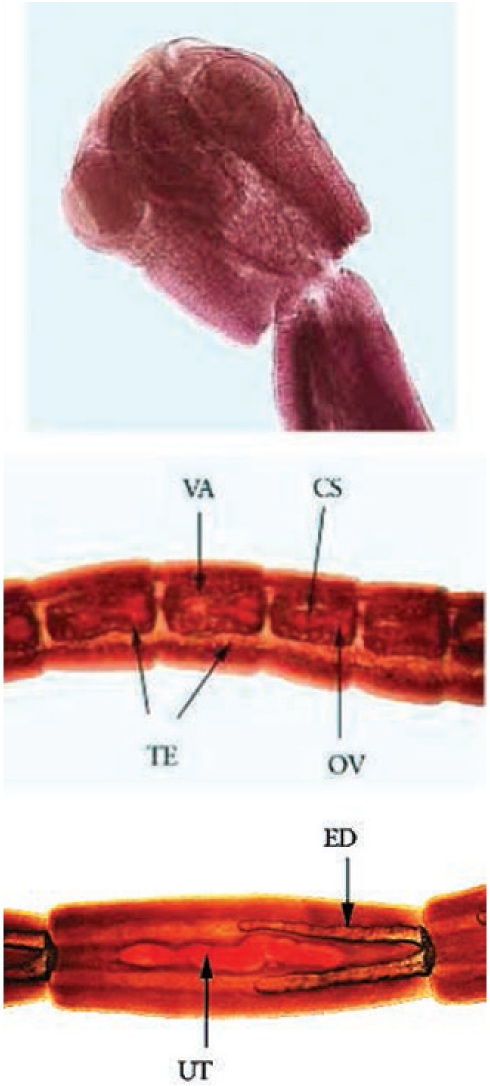


Figure 29.30 *Mesocestoides* spp. (Top) Scolex; two of the suckers are visible with no rostellar hooklets. (Middle) Mature proglottid. CS, cirrus sac; OV, bilobed ovary; TE, numerous testes; VA, vagina. (Bottom) Gravid proglottid. ED, excretory ducts; UT, uterus. (All stained with carmine; courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch29.f30

case was reported in the United States; the patient was a 19-month-old boy from Alexandria, LA. The infection was probably food borne, associated with local dietary customs in the Acadian and Creole communities of Louisiana, including eating sausage containing wild-animal viscera (71). The cases within the United states are probably *Mesocestoides variabilis* (71).

Mesocestoides corti serves as a murine model of human neurocysticercosis caused by *Taenia solium* in terms of the release/secretion of parasite antigens, immune responses elicited within the central nervous system environment, and subsequent pathogenesis. The kinetics of leukocyte subsets infiltrating into the brain have been examined in the context of disruption of the central nervous system barriers at distinct anatomical sites and the mechanisms contributing to these processes. Also, production of various inflammatory mediators and the mechanisms involved in their induction by the Toll-like receptor signaling pathway have been clarified. Overall, the knowledge gained from the mouse model of neurocysticercosis has provided new insights for understanding the events contributing to different stages of this disease and may lead to more effective therapeutic approaches (72).

Taenia crassiceps

Taenia crassiceps is a common tapeworm of the red fox and dogs in the Northern Hemisphere. Larval forms are generally found under the skin and in the body cavities in animals. Human cases are quite rare and appear to depend on the status of the host's immune system. An infection was reported in a 33-year-old patient with AIDS and a history of *Pneumocystis jiroveci* pneumonia and cerebral toxoplasmosis. The patient developed a subcutaneous paravertebral infiltrate that resembled a hematoma. During a period of several weeks, the lesion spread such that almost the entire back was covered. Approximately 3 weeks after admission, the infiltrate ruptured spontaneously, releasing blood and many white spherical masses (2 to 3 mm in diameter). The masses were identified as the larval form of *T. crassiceps*. After therapy with mebendazole and praziquantel, the infiltrate regressed. Praziquantel treatment was continued for 10 weeks, at which time the patient stopped taking the medication. At the end of 4 months, the lesion recurred but was successfully treated with an additional course of mebendazole and praziquantel (73).

A second case in an AIDS patient has been reported from France; this case involved invasion of subcutaneous and muscular tissues by the larval forms of *T. crassiceps* (74). The authors emphasized the problems in differentiating this infection from cysticercosis, coenurosis, sparganosis, and hydatidosis. The proliferative growth of the larvae, recurrence of lesions after surgical removal, and possible invasion of other tissues carry a poor prognosis for this group of immunocompromised patients. The biological properties of *T. crassiceps* cysticerci and the epidemiologic characteristics of pandemic AIDS could indicate the potential for new cases of this disease in humans (75).

Another case illustrates a non-AIDS-associated infection with *T. crassiceps* (76). A healthy 15-year-old girl developed an iridocyclitis in her right eye. A contractible living parasite measuring about 2 mm could be seen in the anterior chamber, while the rest of the eye was not involved

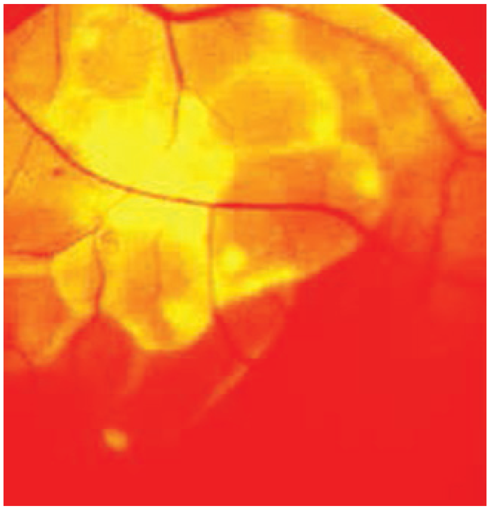
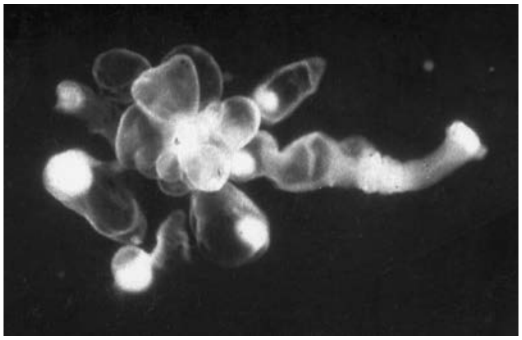


Figure 29.31 *Taenia crassiceps*. Retinal photograph showing budding cysticercus *in situ*. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman; photograph courtesy of Renio Freeman.) doi:10.1128/9781555819002.ch29.f31

(Fig. 29.31). The patient reported having close contact with a young dog in the family. The larval form was removed alive but was not developed enough to have the characteristic scolex (Fig. 29.32). Western blot results provided sufficient indirect evidence to confirm the identity of the organism as *T. crassiceps*. After follow-up for 2 years, no further evidence of ocular or general infestations with this parasite could be found.

Another case was reported in a patient with malignancy (non-Hodgkin's lymphoma) (77). An 82-year-old woman was hospitalized due to progressive pain and swelling in the left forearm and back of the left hand. The symptoms had started 6 weeks earlier after she had fallen on her hand. She sustained soft tissue injury with lymphedema but without fracture. Despite massage therapy, the symptoms worsened. On examination, the patient was febrile (38°C). The left forearm and back of the hand were swollen, and the skin was tender to palpation. The patient had received the last of six cycles of chemotherapy with fludarabine phosphate and cyclophosphamide 2 months before admission. She had undergone radiotherapy of retrocardiac and iliac lymph nodes. Ultrasonography of the arm showed massive edema of subcutaneous tissue and in between muscles and tendons. Multiple, well-defined, cystic lesions were demonstrated intermuscularly. The findings were confirmed by magnetic resonance imaging tomography (Fig. 29.33). On the fifth day after admission, the patient developed a massive swelling of the forearm and an 8-cm lump on the back of the hand. The patient underwent emergency fasciotomy. Jelly-like tissue containing multiple spherical masses with diameters of up to 2 mm, similar to fish eggs, was discovered and removed from the subcutis, muscles, and tendons by multiple excisions. After therapy with praziquantel, the wounds healed, and there were no signs of recurrence for a period of 9 months follow-up.

Figure 29.32 *Taenia crassiceps* cysticercus after surgical removal from the eye. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman; photograph courtesy of Renio Freeman.) doi:10.1128/9781555819002.ch29.f32



The method for obtaining antigenic extracts from *T. crassiceps* cysticerci and their cross-reactivity with *T. solium* cysticerci antigens have made them an interesting antigen source for diagnostic work. The use of 18- and 14-kDa *T. crassiceps* immunoaffinity-purified proteins for detection of anti-cysticercus antibodies in cerebrospinal fluid and/or serum samples by using an enzyme-linked immunosorbent assay system showed good performance and a high specificity for serum samples, dispensing with the use of confirmatory tests, such as immunoblotting, for checking specificity (78).

Trematodes

Alaria americana

Alaria americana is a trematode that is normally found in the intestines of wild mammalian carnivores such as wolves, foxes, raccoons, bobcats, and skunks. The eggs are passed in the stool, hatch in water, and invade freshwater snails of the genus *Helisoma*. Cercariae then leave the snail and infect frog tadpoles. The first case of disseminated human infection by the mesocercarial stage of this worm was found in a 24-year-old man in Canada who died 8 days after the onset of his illness (79). The infection was probably acquired through ingestion of inadequately cooked frogs, which serve as intermediate hosts for this

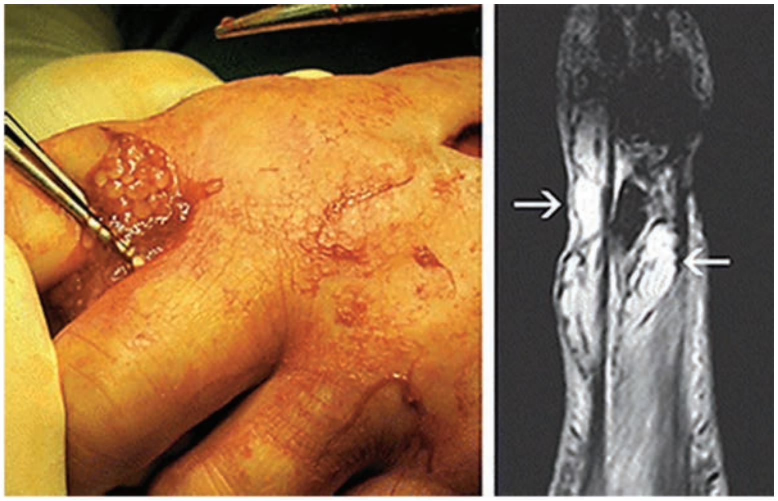


Figure 29.33 *Taenia crassiceps*. (Left) View of the base of the left index finger of the patient during surgery. Numerous fish spawn- or roe-like cysts were found, measuring approximately 3 mm in diameter. At the right upper quadrant a surgical cut in the back of the hand is visible, where further cysticerci were removed. (Right) T2-weighted magnetic resonance imaging tomography of the left forearm. Bright white areas correspond to parasitic infiltration (arrows). (Courtesy of reference 77.) doi:10.1128/9781555819002.ch29.f33

parasite. The infection was diagnosed by lung biopsy, and mesocercariae were found in the stomach wall, lymph nodes, liver, myocardium, pancreas and surrounding adipose tissue, spleen, kidney, lungs, brain, and spinal cord at autopsy (Fig. 29.34 and 29.35). Apparently, there was no host reaction to the parasites. Granulomas were present in the stomach wall, lymph nodes, and liver, but no worms were recovered from these sites.

Two cases of eye infections with mesocercariae of *Alaria* were found in unrelated Asian men who had unilateral decreased vision (80). Both patients had pigmentary tracks in the retina, areas of active and/or healed retinitis, and other signs of diffuse unilateral subacute neuroretinitis (Fig. 29.36). The worms were seen in the retinas and vitreous several years after the original infection was thought to have occurred. The probable source of infection was

Figure 29.34 *Alaria americana*. Living mesocercaria removed from a human liver at autopsy. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman; photograph courtesy of R. S. Freeman.) doi:10.1128/9781555819002.ch29.f34

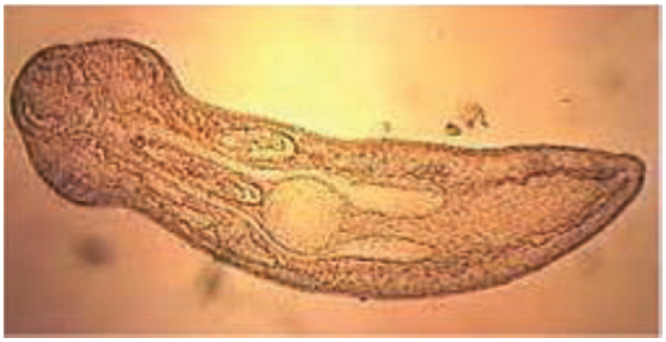


Figure 29.35 *Alaria americana*. (Top left) Mesocercaria in the patient's pons; (Top right) Mesocercaria in lung; (Lower) Magnified image from lung. (From A Pictorial Presentation of Parasites: A cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of R. S. Freeman.)
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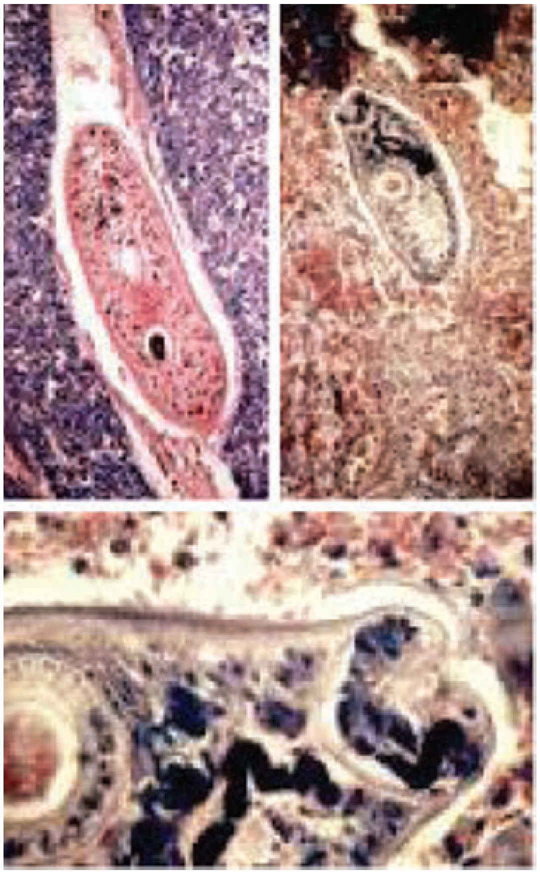


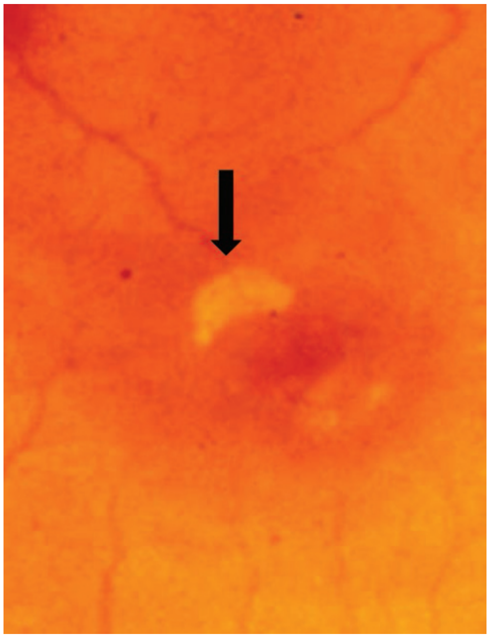


Figure 29.36 *Alaria americana*. Freely moving *Alaria* mesocercaria on the retina of the human eye (arrow). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman; photograph courtesy of R. S. Freeman.) doi:10.1128/9781555819002.ch29.f36

ingestion of undercooked frogs' legs containing the infective stage. In addition to the prolonged intraocular infection, the mesocercariae caused diffuse unilateral subacute neuroretinitis, a condition thought to be caused only by intraocular nematode larvae.

Plagiorchis spp.

Plagiorchis spp. cause human intestinal infections that have been confirmed in the Philippines, Indonesia, Korea, Thailand, and Japan. The infection is acquired from the ingestion of grubs of certain insects thought to be the second intermediate host for this fluke. Ingestion of raw or poorly cooked freshwater fish containing the metacercariae was implicated in the case in Korea (Fig. 29.37) (81). Metacercariae obtained by an artificial ingestion technique used on freshwater fish were administered to a Sprague-Dawley rat; on day 8 postinfection, one *Plagiorchis muris* worm was recovered from the small intestine of the rat. Infections with multiple helminths from northeast Thailand have included *Plagiorchis harinasutai* as well as other unusual trematodes (82). Confirmation of these infections may be difficult since the eggs resemble those of *Opisthorchis viverrini* and *Clonorchis sinensis*.

Plagiorchis vespertilionis is generally considered a bat parasite and was reported for the first time in a human in 2007 (83). The patient was a 34-year-old male who lived in a coastal village in the Republic of Korea. Only one worm, 2.6 mm long and 0.7 mm wide, was recovered after praziquantel therapy and purging with magnesium salts. The fluke characteristics were as follows: a large body size, a sucker ratio of 1:1, a straight cirrus organ, a short distance between the ventral sucker and ovary, well-developed vitellaria, a uterus with descending and ascending loops, and fully developed eggs with an average size of 32.5 × 17.5 μm. The patient had frequently eaten the raw flesh of snakehead mullet and gobies that had been caught near his village.

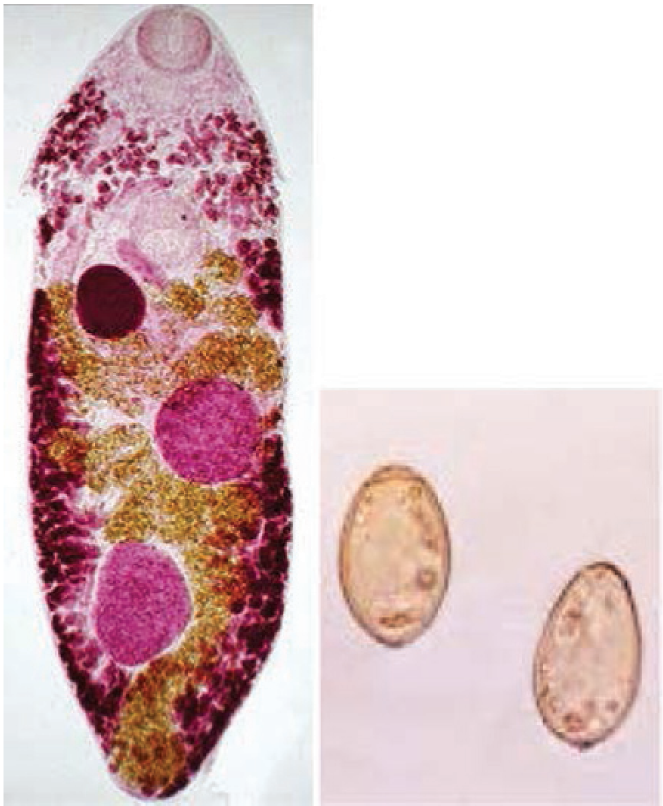


Figure 29.37 *Plagiorchis* spp. (Left) Adult fluke. (Right) Eggs that resemble those of *Opisthorchis viverrini* and *Clonorchis sinensis*. (From reference 81, with permission.) doi:10.1128/9781555819002.ch29.f37

Neodiplostomum seoulense

Neodiplostomum seoulense is known to infect humans in Korea and has been linked to snake consumption. The first human case was found in 1982 in a young man suffering from acute abdominal pain and fever, who had a history of consuming improperly cooked snakes 7 days prior to admission to a hospital (Fig. 29.38) (84). More human

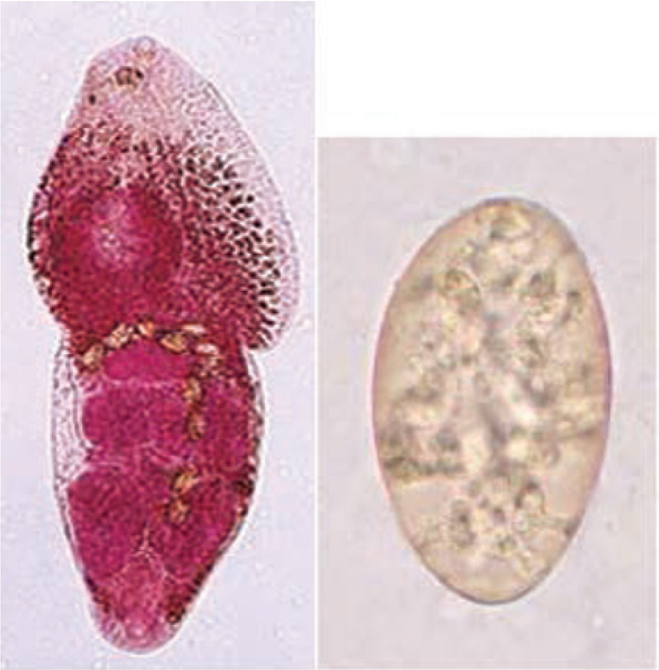


Figure 29.38 *Neodiplostomum seoulense*. (Left) Adult fluke. (Right) Eggs that somewhat resemble those of *Fasciola*, but can be differentiated on the basis of size. (From the Korean Society for Parasitology Web Atlas of Medical Parasitology, http://atlas.or.kr/index.html.) doi:10.1128/9781555819002.ch29.f38

cases were found among soldiers who had eaten raw snakes during their survival training. The parasites can normally be found in house rats, frogs and their tadpoles, and several terrestrial snakes. Symptoms have been noted in human infections and include epigastric pain, diarrhea, fever, and eosinophilia. The eggs can be differentiated from those of *Paragonimus*, *Echinostoma*, and *Fasciola*; they measure 81 to 102 μm long by 51 to 63 μm wide, are golden brown and bilaterally symmetrical, have an oblique opercular margin, and are immature when passed in the stool. In animal studies, this parasite appears to have the potential to kill most strains of mice by causing irreversible damage to their intestine (85). In follow-up studies of intestinal parasitic infections of the Army soldiers in Whachon-gun, Korea, additional cases of infection with *N. seoulense* were identified (86). Additional molluscan intermediate hosts for *N. seoulense* continue to be identified (87).

Spelotrema brevicaeca

Spelotrema brevicaeca has been reported to cause infections in humans in the Philippines. The eggs of these trematodes are suspected of causing lesions in the heart, brain, and spinal cord of patients dying of acute cardiac dilation. Although the complete life cycle is not known, the encysted metacercarial stage has been found in the crab *Cararius maenas*, which is the implicated food source. The adult worms are small, measuring 0.5 to 0.7 mm long by 0.3 to 0.4 mm wide, and have a spinose cuticle. The eggs are quite small (15 to 16 μm long by 9 to 10 μm wide). The infection can be confirmed by finding the eggs in stool or tissues or by finding adult worms in the stool after therapy.

Brachylaima sp.

Two 21-month-old children from the same rural area of South Australia presented 18 months apart with mild abdominal pain and diarrhea; trematode eggs of *Brachylaima* sp. were found in the stool (88). Therapy with praziquantel resulted in cessation of the symptoms and elimination of eggs in the stool. The life cycle involving the common house mouse, poultry, and introduced European helicid snails is well established in South Australia. Both children had been seen eating raw snails; examination of the snails from the environment confirmed that they were infected with metacercariae of this brachylaimid trematode.

In 1998, a 78-year-old woman presented with an 18-month history of intermittent diarrhea. Eggs of a brachylaimid trematode were seen in the stool for a week (Fig. 29.39). After she had been treated with praziquantel, a degenerated adult fluke was recovered from the stool and subsequently identified as *Brachylaima* sp. She lived in South Australia and consumed vegetables grown in her garden, which was infested with helicid snails. These snails, commonly infected with brachylaimid intermediate larval stages, are considered to be the source of human infection (89). This life cycle was established in the laboratory from eggs recovered from human feces in Australia (90). The differential features of *Brachylaima cribbi* n. sp. are the specificity for helicid snails as first and second intermediate hosts; characteristic ventral sucker and body cercarial chaetotaxy; and a long slender adult worm with equal size suckers in the first quarter of the worm, the ventral sucker occupying 41% of the body width, the uterus extending anterior to the ventral sucker, and the vitelline follicles falling short of the posterior margin of the ventral sucker. No other known *Brachylaima* species exhibits all of these features. *B. cribbi* n. sp. is the first brachylaimid known to have infected humans and is probably of European origin, as the intermediate host snails were all introduced into Australia from Europe (91).

Terrestrial edible land snails imported into northeast Spain from Algeria and South Africa and intended for human consumption via sale in public markets were analyzed to assess their contribution to geographic dissemination of *Brachylaima* species. From extensive data analysis, it appears that imported edible *Cornu aspersum* (*Helix*

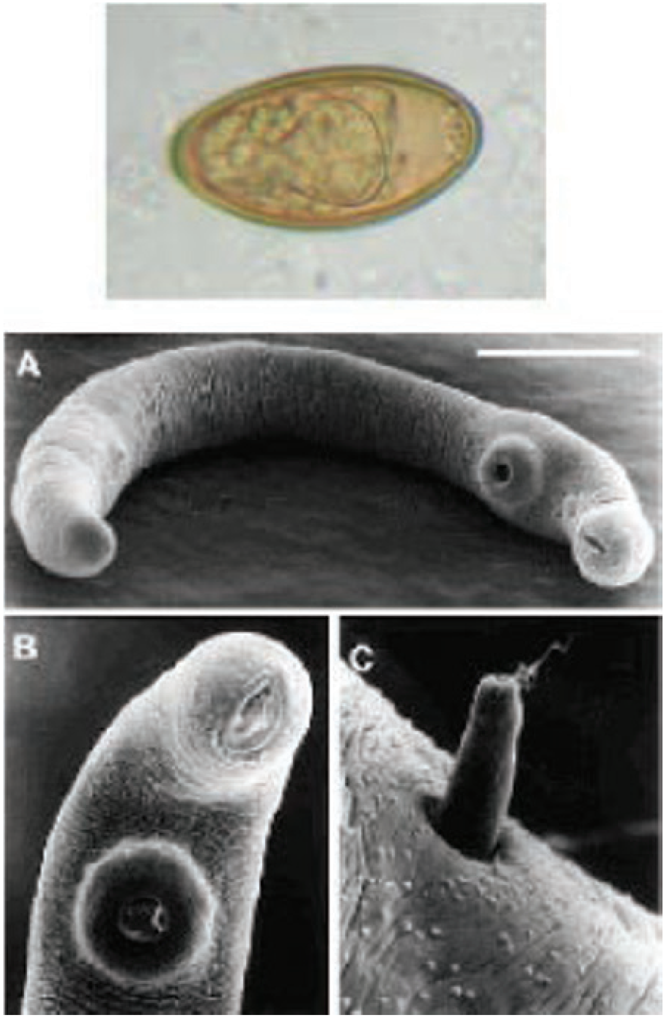


Figure 29.39 *Brachylaima cribbi*. (Upper) Egg; these eggs measure 26 to 32 µm long and 16 to 17.5 µm wide. (Lower) Electron microscopy: (A) Adult *Brachylaima cribbi* trematode (B) has two suckers and a spiny coat and (C) releases sperm from a genital pore. Scale bar, 0.5 mm. (Lower panels from reference 91, with permission.) doi:10.1128/9781555819002.ch29.f39

aspersa) snails could contribute significantly to the geographic spread of *Brachylaima* species by harboring infective metacercariae which could easily infect animals, given their heteroxenous character, and which could be enhanced by commerce in terrestrial snails (92).

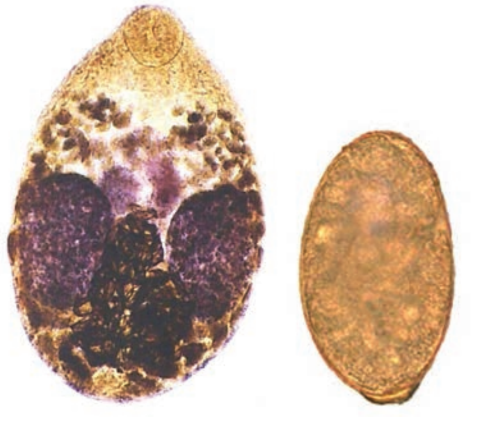
Heliciculture is an excellent alternative to obtain edible snails but its viability is seriously threatened by pathogens, including those involving *Brachylaima* sp. infecting *Cornu aspersum*. Study results confirm the importance of parasites in heliciculture and emphasize the need to prevent the entry of wild reservoirs into the farms and to rapidly remove the carcasses of dead snails from the reproduction units and fattening pens (93).

Nanophyetus (*Troglotrema*) *salmincola*

Human nanophyetiasis is a zoonotic disease recognized in the coastal Pacific Northwest of the United States and is caused by the digenetic trematode *Nanophyetus salmincola*. Ten stool-proven cases from the same geographic area have been identified; five presented with histories of gastrointestinal complaints and five had eosinophilia. Nine of 10 patients had histories of fish ingestion (incompletely cooked or home-smoked salmon or steelhead trout) and were treated with praziquantel. Prompt resolution of symptoms was seen following therapy. In all treated cases, subsequent stool examinations were negative for eggs of *Nanophyetus*. This infection may be most commonly encountered parasitic trematode endemic to North America, and the use of praziquantel in its treatment appears to be efficacious (94).

The trematode *N. salmincola* has been associated with poisoning in dogs eating raw salmon or trout on the Pacific coast of the United States and Canada. Normal hosts would include the coyote, fox, raccoon, mink, and lynx in North America and aborigines of eastern Siberia. The adult worms are quite small, measuring 0.8 to 1.1 mm long by 0.3 to 0.5 mm wide. The eggs are broadly oval, operculated, thick-shelled, and yellow and measure 60 to 80 µm long by 34 to 50 µm wide (Fig. 29.40). When salmon and trout, as well as other fish and the Pacific giant salamander, are eaten raw, metacercariae in their kidneys cause infection in the intestine of the definitive host (the dog). A rickettsia is associated with the fluke infection and has caused severe and fatal symptoms in dogs, coyotes, and foxes; this rickettsia is also possibly a human pathogen.

Figure 29.40 *Nanophyetus (Troglotrema) salmincola*. (Left) Adult fluke, which is quite small and measures 0.8 to 1.1 mm long by 0.3 to 0.5 mm wide. (Right) Egg, which is broadly oval, operculated, thick-shelled, and yellow and measures 60 to 80 µm long by 34 to 50 µm wide. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch29.f40



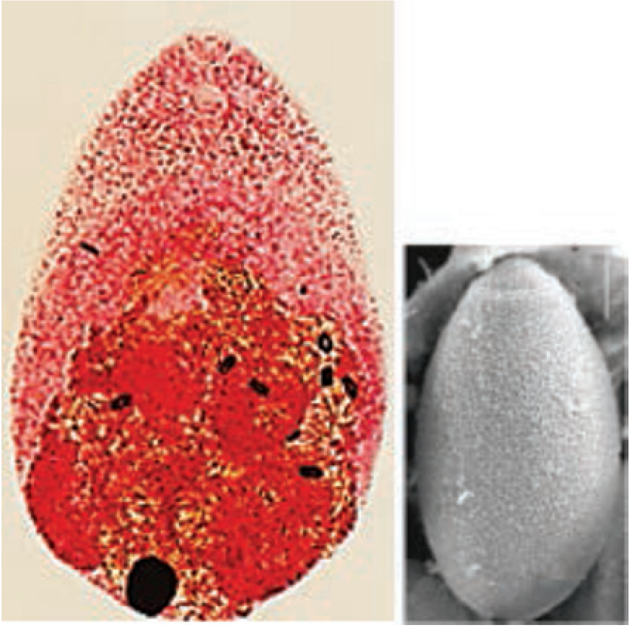
Praziquantel is the recommended therapy for human infection with this trematode.

Stellantchasmus falcatus

Stellantchasmus falcatus infections in two humans have been reported from Korea. Vague abdominal discomfort and hunger pains were reported. In both patients, adult worms were recovered in the stool after treatment with praziquantel (Fig. 29.41) (95–97). Both patients reported eating several kinds of brackish-water fish. Based on these case histories, the total number of human infections remains at 10 to date. This infection has also been documented in a patient eating raw mullet in Hawaii (98). In Thailand, several reports have also shown that *S. falcatus* metacercariae are found in freshwater fish, such as half-beaked fish (*Dermogenus pusillus*) and climbing perch (*Anabas testudineus*), both of which have high infection rates of *S. falcatus* endemic in northern Thailand. Molecular methods of identification have been developed for many of the smaller trematode infections.

Another infection linked to the ingestion of raw mullet is caused by a *Phagicola* sp. A patient in Brazil complained of mild intestinal pain, and examination of the stool specimen revealed trematode eggs in the stool. The patient also had an 8% eosinophilia. After treatment with praziquantel, the symptoms disappeared (99). Another infection linked to the ingestion of raw brackish-water or freshwater fish in Korea is caused by *Heterophyopsis continua*, a trematode whose consumption results in vague abdominal complaints (100).

Figure 29.41 *Stellantchasmus falcatus*. (Left) Adult fluke (courtesy of the CDC Public Health Image Library). (Right) Eggs, SEM view; these eggs measure 26 to 27 µm long and 12 to 13 µm wide with a narrow/small operculum (from reference 95, with permission). doi:10.1128/9781555819002.ch29.f41



Phaneropsolus spinicirrus, *Phaneropsolus bonnei*, and *Prosthodendrium molenkempi*

Members of the *Phaneropsolus* group of trematodes have been found in reptiles, birds, and mammals; however, only *Phaneropsolus bonnei* was reported to have been isolated from humans (Fig. 29.42) (101). Adult worms of *Phaneropsolus spinicirrus* sp. nov. were recovered after treatment with praziquantel followed by a magnesium sulfate purgative 5 h after medication. All stools passed over the next 48 h were preserved in 10% formalin and strained through a 120-mesh screen (200-µm openings), and the strained material was examined with a stereoscopic microscope. The adult worms were stained with Mayer's hemalum, dehydrated, cleared, and mounted for examination. Differences in morphologic characteristics that provided evidence for the identification of *P. spinicirrus* rather than *P. bonnei* included more conspicuous spines on the cirrus, a larger body and internal organs, conical spines on the tegument, an ovoid to bilobed ovary, and a large, V-shaped excretory bladder (102). The structure of the tegumental spines and the characteristic excretory bladder allow the two species to be recognized, even in unstained specimens.

The two small trematodes *Phaneropsolus bonnei* (Fig. 29.43) and *Prosthodendrium molenkempi* have been recovered in large numbers from the intestine of humans; however, there are no well-defined intestinal pathologic or clinical findings. These infections are documented and endemic among rural populations in northeastern Thailand and Laos; in some areas the prevalence is as high as 10 to 40%. Infection occurs after ingestion of raw or poorly cooked small fish contaminated with infected naiads (water nymphs of dragonflies). Diagnosis can be confirmed by finding adult worms or eggs in the stool; however, the eggs may be difficult to differentiate from each other and from those of *O. viverrini*. *O. viverrini* and several species of heterophyid and lecithodendriid flukes, which are endemic in two riverside localities in Laos, suggesting that the intensity of infection and the relative proportion of fluke species vary by locality along the Mekong River basin (103). Staining using a 1% (wt/vol) concentration of potassium permanganate for 1 min is useful in differentiating the egg surface morphology of *O. viverrini*, *Haplorchis taichui*, and *P. bonnei* eggs (104).

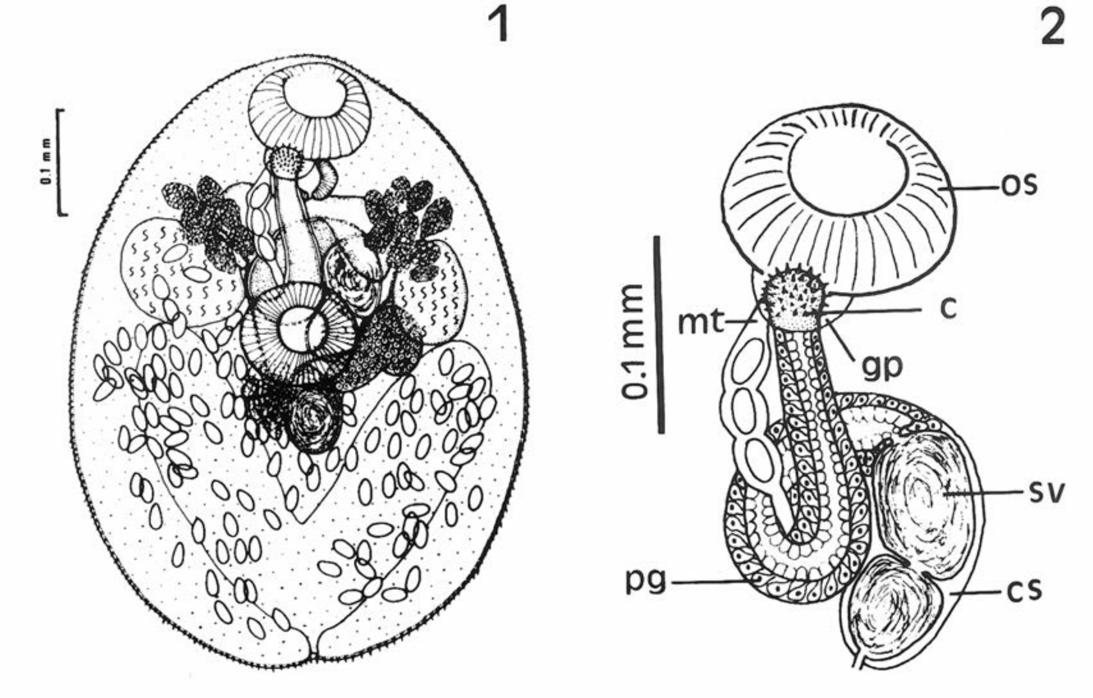


Figure 29.42 *Phaneropsolus spinicirrus* isolated from a patient. (1) Ventral view of the holotype. (2) Ventral view of the structure of the terminal portion of the reproductive system. c, cirrus; cs, cirrus sac; gp, genital pore; mt, metraterm; os, oral sucker; pg, prostate gland, sv, seminal vesicle. (Reprinted from reference 102 with permission.) doi:10.1128/9781555819002.ch29.f42

Haplorchis taichui

Haplorchis taichui is a minute human intestinal trematode that parasitizes the small intestine of humans and other mammals (Fig. 29.44). It is the smallest trematode reported from the Republic of Korea. Pathologic changes seen in three patients from Thailand include mucosal ulceration, mucosal and submucosal hemorrhages, fusion and shortening of the villi, chronic inflammation, and fibrosis of the submucosa (105).

An exceptionally high rate of infection (36%) has been identified in human populations on Mindanao Island, southern Philippines, and Lao PDR. The source of infection has been linked to consumption of raw or undercooked freshwater fish containing infective metacercariae. Clinical symptoms include upper abdominal discomfort or pain and loud gurgling noises produced by the movement of gas, fluid, or both in the alimentary canal, sounds which are audible at a distance. Other reported symptoms were nausea, chronic diarrhea, and weight loss. Symptoms rapidly resolved after treatment with praziquantel (106). The adult fluke is pyriform; eggs and the ventral sucker can generally be seen (106). Molecular testing has made detection of these small trematodes much easier.

Gymnophalloides seoi

Gymnophalloides seoi is a new human intestinal trematode that was first found in a Korean woman who complained of epigastric discomfort. This trematode is highly prevalent among the inhabitants of a seashore village on a southwestern island of the Republic of Korea. Normally, it is a parasite of shorebirds and is thought to be the only known species in the family *Gymnophallidae* that can infect humans. Various bivalves (clams) are known to serve as the first or second intermediate hosts for some of these trematodes. Symptoms are generally somewhat nonspecific and include abdominal pain, diarrhea, indigestion, anorexia, easy fatigability, and weakness. On the basis of fecal examinations, 70 (71.4%) of 98 patients were found to be infected with various intestinal parasites. *G. seoi* eggs were found in 48 (49.0%) of 98 patients. Individual worm burdens, as measured by collection of adult flukes after treatment and purgation, ranged from 106 to 26,373 specimens. The average number of worms per patient was 3,326. There were no sex- or age-related differences. This study indicates that *G. seoi* is a human intestinal trematode under natural conditions and does not represent just an accidental infection (107, 108). The eggs of *G. seoi* mea-

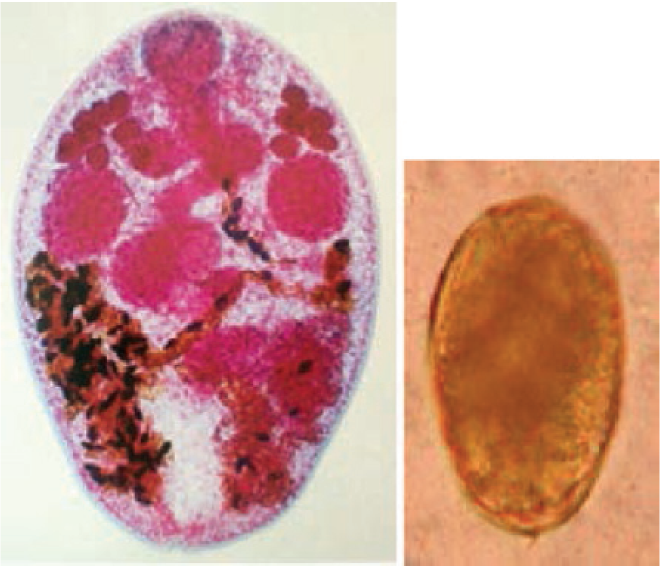
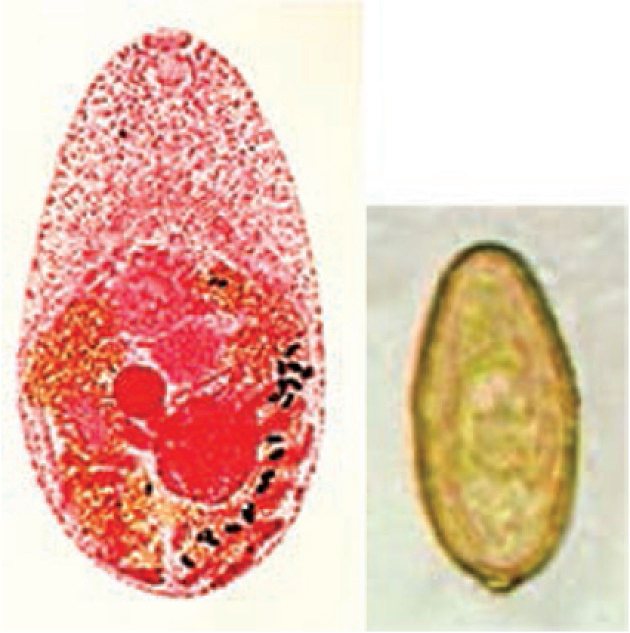


Figure 29.43 *Phaneropsolus bonnei*. (Left) Adult fluke, (Right) Eggs, which are quite small and measure 23 to 33 µm long by 12 to 18 µm wide. (Courtesy of Department of Parasitology, Faculty of Medicine, Chulalongkorn University, http://www.cai.md.chula.ac.th/lesson/test/5403/html/04morp.html.) doi:10.1128/9781555819002.ch29.f43

Figure 29.44 *Haplorchis taichui*. (Left) Adult fluke. (Right) Egg, which measures 28 by 14 µm. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch29.f44



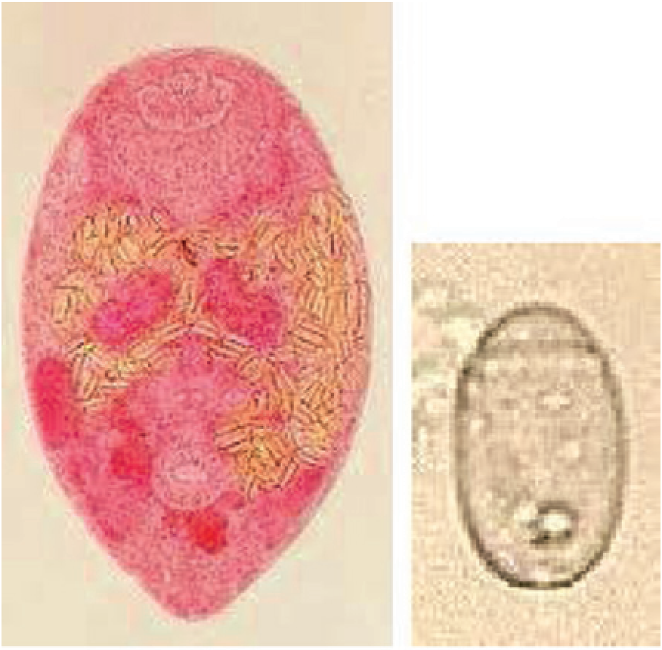


Figure 29.45 *Gymnophalloides seoi*. (Left) Adult fluke, which measures 0.4 to 0.5 mm by 0.2 to 0.3 mm—the smallest of all human trematodes reported in the Republic of Korea (courtesy of the CDC Public Health Image Library). (Right) Egg, which measures from 20 to 25 µm to 11 to 15 µm (from reference 107, with permission). doi:10.1128/9781555819002.ch29.f45

sure 19 to 25 µm in length (smaller than *C. sinensis*) and have a thin, transparent shell (Fig. 29.45). The adult worm measures approximately 0.4 mm long and 0.25 mm wide (Fig. 29.45).

Metorchis conjunctus (North American Liver Fluke)

A common-source outbreak of acute clinical illness among 19 people who ate raw fish (sashimi) prepared from the white sucker has been reported from Canada. The sashimi contained infective metacercariae of *Metorchis conjunctus* (Opisthorchiidae). The adult fluke lives within the biliary tree of the definitive host (Fig. 29.46). Symptoms included persistent upper abdominal pain, low-grade fever, high eosinophil counts, and increased liver enzyme levels with an obstructive pattern. After 15 days, trematode eggs were recovered in the stool specimens; morphologically they were identical to those of *O. viverrini*. The symptoms lasted several weeks, but the patients responded rapidly to therapy with praziquantel. Necropsy of golden hamsters that had been fed metacercariae from the fish revealed adult flukes identified as *M. conjunctus*. Apparently, this is a newly emerging human disease and represents the first report of a common-source outbreak of an acute illness

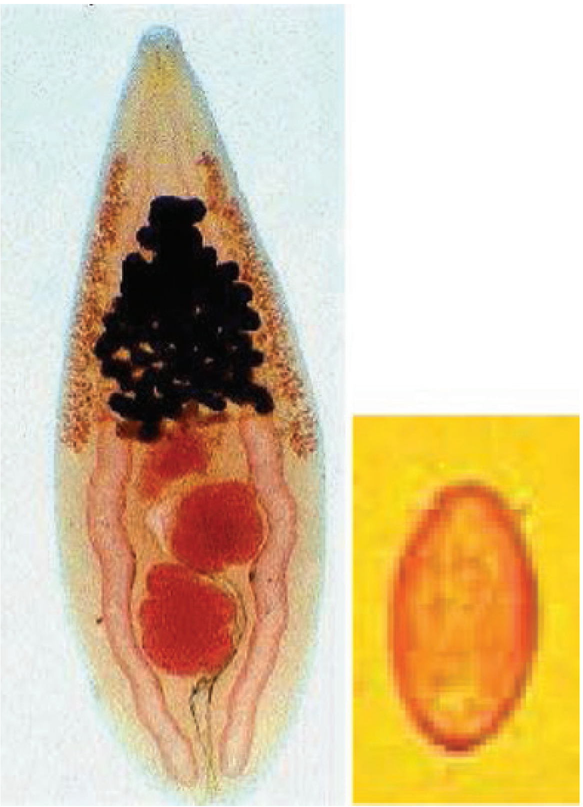


Figure 29.46 *Metorchis conjunctus*. (Left) Adult fluke (courtesy of University of Sydney, Centre for Veterinary Education, http://vetbook.org/wiki/dog/index.php/Metorchis_spp). (Right) Egg with very similar morphology to many of the other small trematodes (courtesy of McGill University/Canada, on Stanford's site, http://www.stanford.edu/group/parasites/ParaSites2006/Metorchiasis/diagnostics_management&therapy.html). doi:10.1128/9781555819002.ch29.f46

caused by these North American liver flukes (109). Although some patients remain asymptomatic, the prevalence in some isolated communities within Canada reaches 20% (110). Because fish parasites infecting humans within the United States and Canada are becoming more important and prevalent, a composite list has been provided (Table 29.2).

It is important to remember not only that opisthorchiid and heterophyid eggs are similar, but that great variability is present among eggs from the same species. Without evidence of the adult fluke or molecular diagnostic options, confirmation to genus/species can be difficult.

TABLE 29.2 Human parasites acquired from the ingestion of raw or poorly cooked fish[a,b]

Parasite	Fish host(s)
Nematodes	
Anisakis simplex	Salmonids, tuna, herring, mackerel, squid
Pseudoterranova decipiens	Cod, pollack, haddock
Eustrongylides spp.	Killifish, estuarine species
Dioctophyma renale	Freshwater fish, estuarine species
Cestodes	
Diphyllobothriurn latum	Salmonids, pike, perch, burbot
Diphyllobothrium dendriticum	Salmonids
Diphyllobothrium ursi	Salmonids
Diphyllobothrium spp.[c]	Salmonids
Schistocephalus solidus	Sticklebacks
Trematodes	
Troglotrema (*Nanophyetus*) *salmincola*	Salmonids
Metorchis conjunctus	White sucker
Haplorchis taichui	Freshwater fish
Plagiorchis spp.	Freshwater fish
Cryptocotyle lingua	Flounder
Amphimerus pseudofelineus	Freshwater fish

[a] Adapted from reference 110.

[b] Not every parasite mentioned in this table has been included in this chapter on unusual parasites; often case reports are very limited. Parasites listed in this table are geographically relevant to the United States and Canada, and the table does not include all parasites discussed in the chapter.

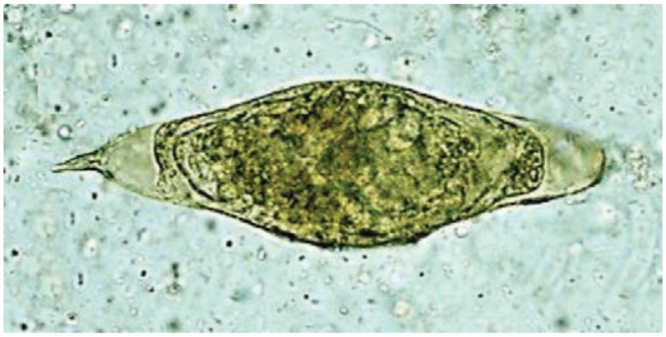
[c] The taxonomy of the genus *Diphyllobothrium* remains unclear.

Schistosoma mattheei

Schistosoma mattheei causes infections in sheep, cattle, and horses; human infections, which caused mild disease, have also been reported from South Africa. The eggs resemble those of *Schistosoma intercalatum*, have a terminal spine, and measure 120 to 180 µm in length (Fig. 29.47). Clinical specimens include urine and stool; eggs can be recovered in both. Although human infections tend to be mild, the effect of this infection on animals is more serious.

The pathology of human disease is similar to that seen in other schistosome diseases and can include egg granulomas in the large intestine and liver, mild hepatosplenomegaly, and granulomatous bowel disease. There tends to be coinfection with either *Schistosoma haemato-*

Figure 29.47 *Schistosoma mattheei*. Image of *Schistosoma intercalatum* egg (very similar to *S. mattheei*). doi:10.1128/9781555819002.ch29.f47



bium or *Schistosoma mansoni*. Patients may present with mild mucoid diarrhea in which blood is present, crampy abdominal pain, and general nonspecific complaints; many patients remain asymptomatic. Praziquantel is recommended for therapy.

Enzyme electrophoresis indicates that most, if not all, *S. mattheei* eggs passed in the urine in human infections derive from *S. mattheei* females *in copulo* with *S. haematobium* males. It appears that *S. mattheei* males do not reach sexual maturity in humans; however, the *S. haematobium* × *S. mattheei* males possibly do (111). Studies indicate that it is highly unlikely that *S. mattheei* and *S. haematobium* will evolve into a single species; it does not seem likely that the virulence of the parental species will be influenced (112).

Philophthalmus lacrimosus

Philophthalmus lacrimosus is an avian eye trematode, found primarily in the eyes of wild and domestic birds. A number of mammals, including humans, can serve as the intermediate as well as the definitive host; however, human cases are quite rare. In cases of conjunctivitis, one of the symptoms is the feeling of having a foreign body in the eye. In one patient from Mexico, a small live parasite was found in the connective tissue of the conjunctiva and was removed surgically (Fig. 29.48) (113). Another case has been reported from Thailand (114).

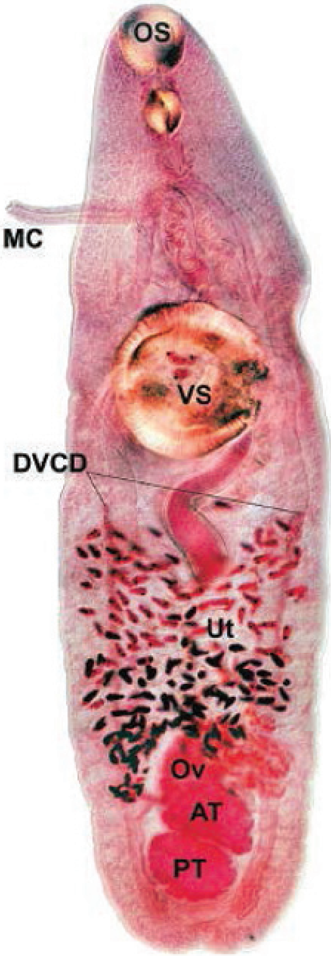


Figure 29.48 Adult *Philophthalmus lacrimosus* trematode. OS, oral sucker; MC, muscular cirrus; VS, ventral sucker; DVCD, distal vitellaria collecting duct; Ut, uterus; Ov, ovary; AT, anterior testis; PT, posterior testis. (Courtesy of Pinto RM et al, *Mem Inst Oswaldo Cruz* **100**:579–583, 2005.)
doi:10.1128/9781555819002.ch29.f48

Achillurbainia spp.

Achillurbainia spp. are trematodes (similar to *Paragonimus* spp.) that are normally found in the maxillary sinuses of the opossum. They have been isolated from humans. In one case of surgical inguinal hernia repair, massive numbers of small granulomata on the omentum and other peritoneal surfaces were found to contain trematode eggs like those of *Achillurbainia recondita* (115). *Achillurbainia* eggs and/or adult worms have been found in retroauricular cysts or abscesses in individuals in West Africa and Southeast Asia. Another case was reported from the United Kingdom in a 29-year-old Nigerian student (116). Trematode eggs were found in a neck lump aspirate that resembled *Paragonimus uterobilateralis* or *Achillurbainia*; based on clinical findings, *Achillurbainia* was chosen for the diagnosis.

Pentastomids

Armillifer spp., *Linguatula serrata*, and *Sebekia* spp.

The organisms are found in a separate phylum, Pentastomida, and are called tongue worms. Human infections have been reported from Africa, Europe, Asia, and the Americas (117). A representative life cycle is seen in Fig. 29.49. The pentastomids are a group of parasites classified between the arthropods and annelids (118).

When humans serve as the intermediate hosts, the infective larvae die *in situ*. However, when mature larvae (often encysted) are ingested, they may migrate from the stomach, attach themselves to nasopharyngeal tissues, develop into adult pentastomids, and produce symptoms of the halzoun syndrome. Symptoms include throat discomfort, paroxysmal coughing, sneezing, and occasionally dysphagia and vomiting (119).

Occasionally, pentastomid larvae infect the eye. Inflammation is minimal, and infection is probably the result of direct eye contact with water containing pentastomid eggs (120). In one case report, a 34-year-old woman in

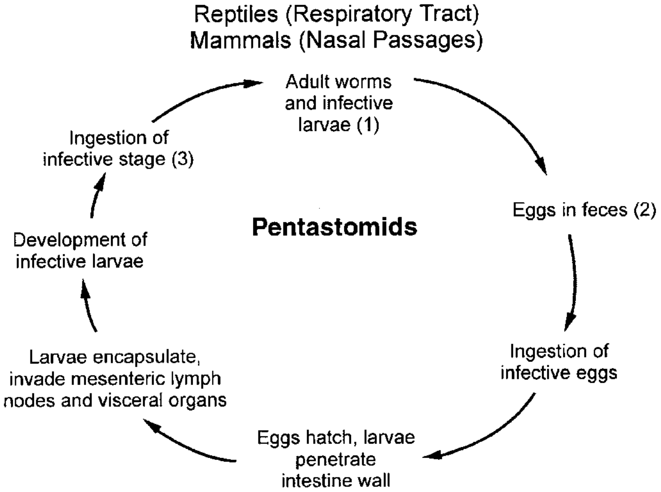

Reptiles (Respiratory Tract)
Mammals (Nasal Passages)
Adult worms and infective larvae (1)
Eggs in feces (2)
Ingestion of infective eggs
Eggs hatch, larvae penetrate intestine wall
Larvae encapsulate, invade mesenteric lymph nodes and visceral organs
Development of infective larvae
Ingestion of infective stage (3)
Pentastomids

Figure 29.49 Life cycle of the tongue worms (pentastomids) *Armillifer* and *Linguatula* spp. (1) Humans ingest raw or inadequately cooked snake or lizard; (2) humans ingest eggs on vegetation or in water; (3) humans ingest the flesh of intermediate hosts containing infective larvae.
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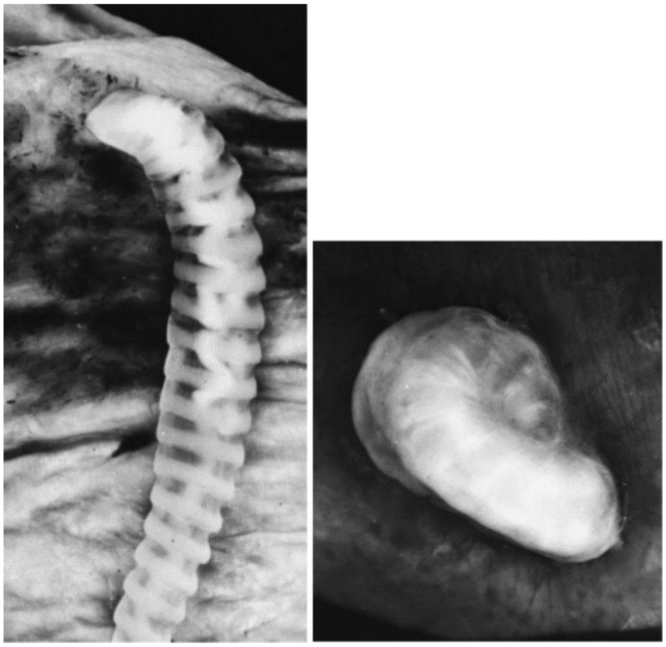


Figure 29.50 (Left) Nonencysted mature *Armillifer armillatus* larva attached to the abdominal surface of a human diaphragm (magnification, ×4). (Right) Encysted *Armillifer armillatus* larva on the surface of the liver of a 55-year-old Zairian. The cyst contains a C-shaped larva (magnification, ×12). (Armed Forces Institute of Pathology photographs.)
doi:10.1128/9781555819002.ch29.f50

Guayaquil, Ecuador, presented with a 2-month history of ocular pain on the right side, with conjunctivitis and visual difficulties due to the presence of a shadow (121). Apparently, when she lay down on her right side, her vision became clear. Examination revealed a mobile body in the anterior chamber of the eye; the body was removed, fixed, stained, and mounted for more detailed microscopic examination. The parasite was identified as a third-stage instar larval form of *Linguatula serrata*. This case represents the first report in Ecuador and South America.

A case of dermatitis in a 31-year-old Costa Rican woman was reported to be caused by a pentastomid larva belonging to the genus *Sebekia*, apparently the first reported case of infection of a human by this genus. The adult worms are parasites found in the respiratory tract of reptiles, and the larval forms are found in the viscera and muscles and along the spinal cord of freshwater fish. The patient, who was pregnant, presented with a "strong itch" in the lower right quadrant of her abdomen and a "biting sensation," particularly at night. She had a serpiginous burrow surrounded by an intense erythematous zone on her lower abdomen and a 30% eosinophilia. A white, active larva approximately 1 cm long was removed from the burrow. On the basis of morphologic features, including spines on the hooks and other details, the larva was identified as being in the genus *Sebekia*. Although there is no way to determine how the patient became infected or why the larva migrated to the skin as does larva migrans, the possibility of ingesting water containing eggs of the parasites or ingesting raw or improperly cooked fish could explain the infection (122).

Diagnosis is made by identifying the pentastomid in a biopsy specimen or at autopsy (Fig. 29.50 and 29.51). Treatment is usually not necessary; however, surgical removal of the parasite is recommended if the patient is symptomatic.

Acanthocephalans

Macracanthorhynchus hirudinaceus and *Moniliformis moniliformis*

The acanthocephalans (thorny-headed worms) were formerly included in the same phylum as the nematodes. These worms get their name from the proboscis, which is covered with spines. The life cycle is seen in Fig. 29.52 and adult worms are seen in Fig. 29.53.

Very few cases have been reported in the literature; however, reported symptoms include abdominal pain and tenderness, anorexia, and nausea. In some cases, adult worms were passed in the stool (Fig. 29.54) (123, 124).

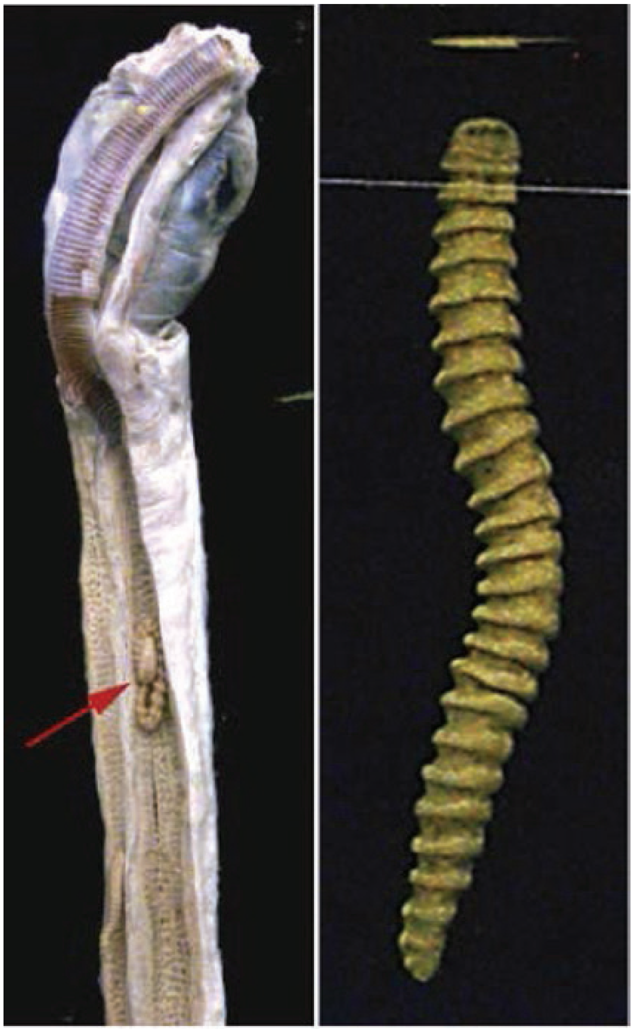


Figure 29.51 (Left) Worm-like adults of *Pantastomum* sp. in the trachea of a snake. (Right) Pentastome larva with marked external annulations. (Courtesy of the Natural History Collection, The University of Edinburgh, http://www.nhc.ed.ac.uk/index.php?page=24.25.333.374.) doi:10.1128/9781555819002.ch29.f51

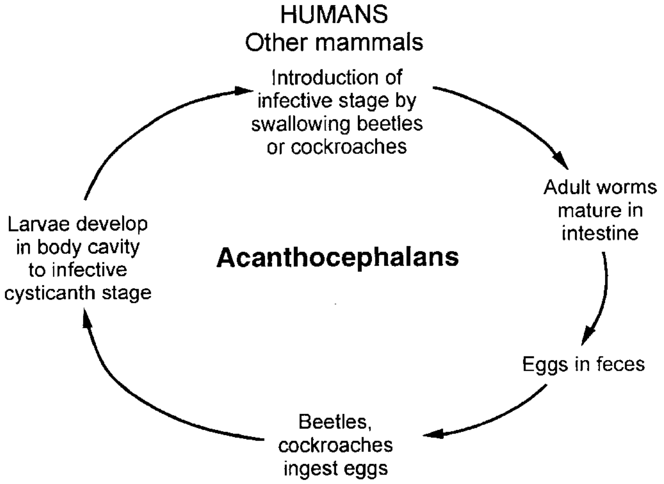
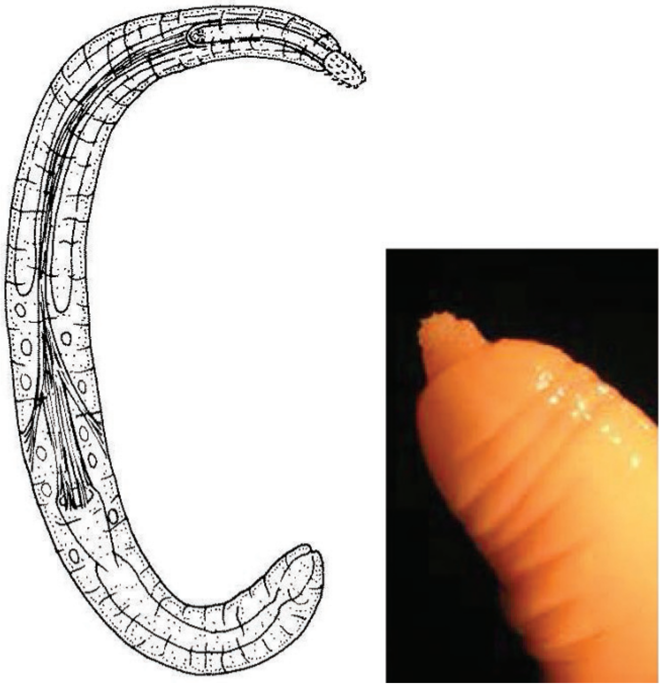

HUMANS
Other mammals
Introduction of infective stage by swallowing beetles or cockroaches
Adult worms mature in intestine
Acanthocephalans
Eggs in feces
Beetles, cockroaches ingest eggs
Larvae develop in body cavity to infective cysticanth stage

Figure 29.52 Life cycle of the acanthocephalans (thorny-headed worms). doi:10.1128/9781555819002.ch29.f52

In the past few years, a number of human infections with *Macracanthorhynchus hirudinaceus* have been reported from China. The infections have been primarily in children, probably acquired from the ingestion of raw beetles. Clinical manifestations include intestinal obstruction and perforation, with perforation occurring in over 200 cases. No eggs were found in the stool specimens of these patients. In many cases, the worms were recovered at surgery or autopsy. Signs and symptoms of infection with *Moniliformis moniliformis* include serious gastrointestinal pain, diarrhea, exhaustion, somnolence, and tinnitus; vomiting, jaundice, cough, and a protuberant abdomen have also been reported (125). In children, the infection has resulted in retarded development and irritability (38).

In some areas of China, eggs have been recovered in 16 to 60% of pigs examined (38); in pigs, this worm can be found worldwide. In Fig. 29.55, the proboscis of *M. hirudinaceus* is seen attached to the pig bowel wall. The

Figure 29.53 (Left) Thorny-headed worm. Note the proboscis, which is covered with spines. (Illustration by Sharon Belkin.) (Right) Close-up image of the anterior end of the worm and the proboscis containing hooks (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch29.f53



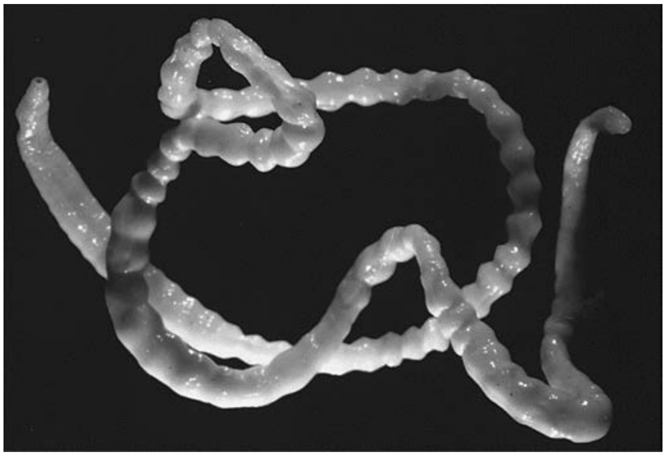


Figure 29.54 *Moniliformis moniliformis*, complete adult specimen, approximately 133 mm long (magnification, ×5) (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch29.f54

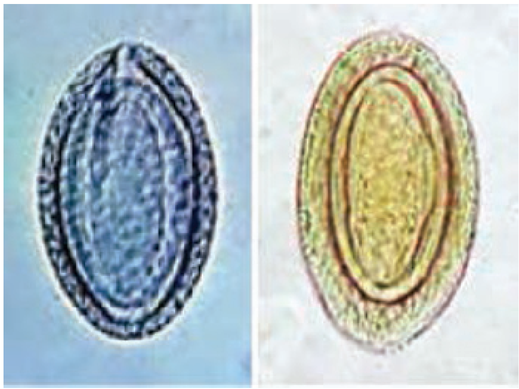


Figure 29.56 *Macracanthorhynchus hirudinaceus* eggs. These eggs measure 80 to 100 µm by 50 µm, have a thick, dark brown, textured shell, and contain a developed acanthor larva with hooks. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch29.f56

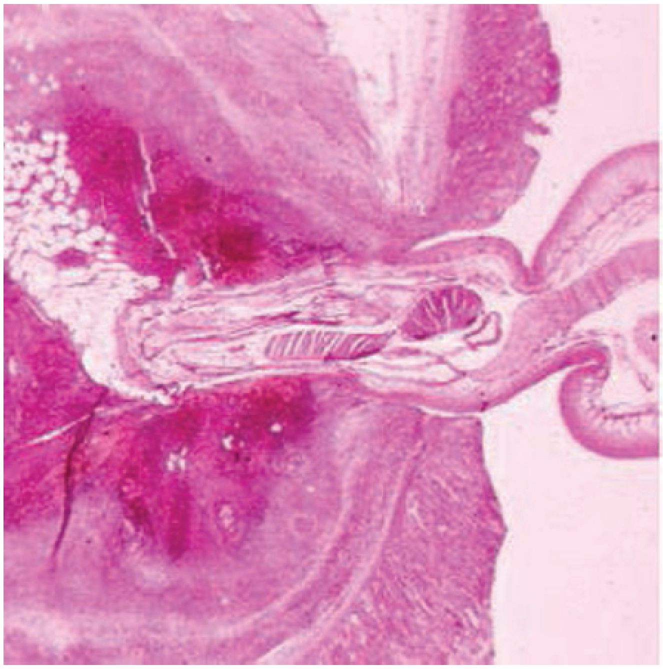


Figure 29.55 *Macracanthorhynchus hirudinaceus* proboscis attached to pig bowel wall. The insertion of the toothed proboscis, along with the inflammation, ulceration, and hemorrhage caused, is seen in this low-power view. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch29.f55

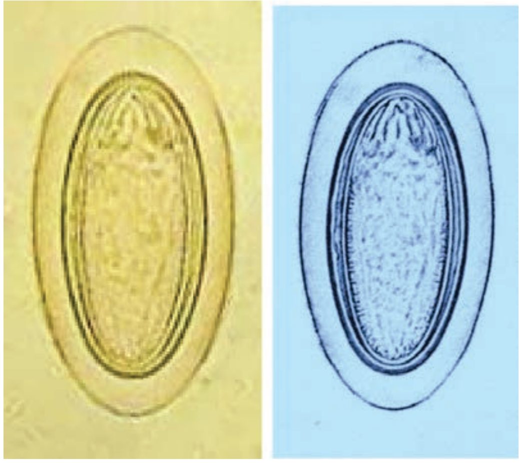


Figure 29.57 *Moniliformis moniliformis* eggs. These eggs measure 90 to 125 µm by 65 µm and contain a fully developed acanthor larva with visible hooks. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch29.f57

eggs of *M. hirudinaceus* and *M. moniliformis* can be seen in Fig. 29.56 and 29.57. Both eggs contain a fully developed acanthor larva, and the rostellar hooks are also visible.

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Parasitic Infections in the Compromised Host

Entamoeba histolytica
Free-living amebae
Blastocystis spp.
Giardia lamblia
Toxoplasma gondii
Cryptosporidium spp.
Cyclospora cayetanensis
Cystoisospora (*Isospora*) *belli*
Sarcocystis spp.
Microsporidia
Leishmania spp.
Strongyloides stercoralis
Plasmodium spp.
Babesia spp.
American trypanosomiasis
Crusted scabies

This chapter discusses some of the representative opportunistic organisms that can cause disease in immunocompromised patients. Any parasitic infection in the immunocompromised host may cause more severe symptoms; however, the organisms presented in this chapter have been identified as causing severe disease in this population.

The body has three types of host defense mechanisms: surface and mechanical factors, the humoral immune system, and the cellular immune system. Within these three groups, further distinction can be made between first-line (nonspecific) innate immunity and second-line (specific) adaptive immunity. By definition, a compromised host is one in whom normal defense mechanisms are impaired (e.g., AIDS), absent (e.g., congenital deficiencies), or bypassed (e.g., penetration of the skin barrier). These patients are becoming more common in most medical facilities and represent a growing problem in terms of diagnosis and subsequent therapy.

As seen in Table 30.1, first-line surface and mechanical factors include the skin, the cough reflex, ciliary motion (respiratory epithelium), and flushing action (urine); secondary defense mechanisms include immunoglobulins such as IgA. In the humoral portion of the immune system, first-line defenses include complement, lysozyme, fibronectin, and interferon; secondary defense mechanisms include antibodies such as IgM and IgG. The cellular portion of the immune system includes first-line defenses such as phagocytes (polymorphonuclear leukocytes [neutrophils and eosinophils] and monocytes) and natural killer cells (T cells); the second line of defense is defined as cell-mediated immunity. Specific problems and potential causes that can arise include penetration of the skin barrier (needle penetrations, burns); splenectomy (impaired IgM antibody production); aspiration of stomach contents, impaired cough reflex, impaired microbicidal clearance by alveolar macrophages, defects in complement activity, etc. (alcoholism); neutropenia (use of immunosuppressive drugs); problems with inhibited phagocytosis (rheumatic diseases); neutropenia, cell-mediated immunity defects, and antibody response defects (renal failure); skin and mucous membrane defects due to poor protein production as well as diminished IgA levels in the gut (malnutrition); and diminished humoral and cellular immunity defenses (AIDS, other underlying infectious diseases). To determine possible host defense defects, a number of procedures can be used (Table 30.2).

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TABLE 30.1 Host defense mechanisms

Immunity type	Factor		
	Surface and mechanical	**Humoral (antibody dependent)**	**Cellular (antibody independent)**
Nonspecific	Skin (intact) (intravenous lines, obstruction of ureters, bronchi) Cough reflex Ciliary motion in respiratory epithelium Flushing action (urine)	Complement Lysozyme Fibronectin Interferon	Phagocytes (neutrophils) Chemotaxis Phagocytosis (monocytes, eosinophils) Natural killer cells
Specific	Immunoglobulins (IgA) (antibody found in secretions)	Antibodies B cells (IgG, IgM) (found in serum and extravascular spaces) IgE (affinity for mast cells, basophils, and eosinophils)	Cell-mediated immunity Sensitized cells (cytotoxic T cells, activated macrophages, natural killer cells)
Additional comments		IgG and IgM fix complement; initiate complement-mediated lysis of the target cell. IgG and IgE bind to macrophages, eosinophils, and mast cells via the Fc portion and to the target cell via the Fab portion	Macrophage functions include detection of organism invasion; restriction of organism spread; recruitment of immune cells; action as accessory cells in lymphocyte activation and as effector cells in cell-mediated immunity

Humans have very effective defense mechanisms to protect themselves against foreign invaders. One system consists of surface and mechanical barriers, pH, temperature, phagocytosis, and nonspecific inflammation (innate resistance). The other system is induced and includes specific products that recognize foreign invaders (adaptive immunity). The two major components of the adaptive immune response are humoral (antibody) and cellular (sensitized cells); B lymphocytes (B cells) are responsible for the humoral response, and T lymphocytes (T cells) are responsible for the cellular response. The human immune system is very complex, with different classes of antibody and various subsets of T cells that have different functions as immune effectors. Unfortunately, the immune system is not always protective and under certain circumstances can be directed toward the human body itself, not the foreign invader.

Within the context of this background information on the immune system, various parasitic infections are discussed, with emphasis on infections in the immunocompromised patient. With the continued increase in the number of these patients, every institution will eventually provide medical care for someone in this category and

TABLE 30.2 Selected procedures for determination of host defense defects[a]

Defect	Procedure	
	Screening	**Diagnostic**
Inflammation	White blood cell count and differential C_{50} hemolytic complement	Complement levels Inhibitor levels Complement-fixing immune complexes
Phagocytosis	White blood cell count and morphology Immunophenotyping	Phagocytic index Bactericidal tests Nitroblue tetrazolium test Cell surface glyproteins Lymph node biopsy
Humoral antibody	Immunoelectrophoresis Immunoglobulin levels in serum	IgE levels in serum Secretory IgA B-cell levels, subsets Isohemagglutinins Purine-metabolizing enzymes
Cellular sensitivity	Skin tests Quantitation of T cells and T-cell subsets	Sheep erythrocyte rosettes Mitogen responses Lymph node biopsy CT, MRI of thymus

[a] Adapted from Sell S, *Immunology, Immunopathology, and Immunity*, 6th ed, ASM Press, Washington, DC, 2001.

laboratories will continue to perform more procedures for the diagnosis of opportunistic infections.

Routine diagnostic procedures for the diagnosis of parasitic infections in the compromised host are presented in Table 30.3, and clinical findings in normal and compromised hosts are described in Table 30.4. Molecular methods for diagnosis continue to be developed, including some newer gastrointestinal panels. A 19-plex laboratory-developed gastrointestinal pathogen panel (GPP) using Luminex xTAG analyte-specific reagents (ASRs) has been validated (xTAG Gastrointestinal Pathogen Panel; Luminex, Inc., Austin, TX), which simultaneously screens directly in fecal specimens for diarrhea-causing pathogens, including bacteria, viruses, and parasites (*Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*). The BioFire FilmArray Gastrointestinal Panel is another FDA-approved multiplex PCR test that can simultaneously detect 22 gastrointestinal pathogens in about an hour (BioFire FilmArray Gastrointestinal Panel; BioFire Diagnostics, Salt Lake City, UT). The panel includes *Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, and *Giardia lamblia*, along with a number of bacteria and viruses.

Parasitic infections in individuals with a normal immune system are not uncommon. Not only are they unpleasant and debilitating, but they can be fatal. However, individuals with immune system defects have an abnormally high susceptibility to infections with nonvirulent and minimally pathogenic organisms. Many of these individuals contract parasitic infections in addition to suffering numerous infectious episodes with bacterial, viral, and fungal organisms. Immune system deficiencies can be attributed to congenital absence, abnormal development, malignancy, therapy with cytotoxic drugs, irradiation, or infections, such as with HIV. Of particular interest are patients with AIDS.

Although any parasitic infection may be more severe in the immunosuppressed host, certain organisms tend to produce greater pathologic sequelae in these patients, while other organisms occur with a higher frequency in individuals with certain immune system deficiencies. It is important for the laboratorian and clinician to be aware of problems that these organisms can cause in compromised patients and to be aware of the proper diagnostic techniques and their clinical relevance.

Entamoeba histolytica

Entamoeba histolytica is the cause of amebiasis (Tables 30.3 and 30.4; Fig. 30.1). Two types of disease, reviewed here, are particularly helpful when one is discussing serologic test results. "Intestinal disease" indicates that the organisms are confined to the gastrointestinal tract, with no gross or microscopic invasion through the mucosal lining. "Extraintestinal disease" denotes invasion of the mucosal lining of the gastrointestinal tract, the organisms having passed through the mucosal lining, entered the bloodstream, and been carried to other body tissues, particularly the liver. In patients in whom the organisms have begun to invade the mucosal lining, interpretation of serologic tests may be difficult. In this situation, the terms "intestinal" and "extraintestinal" do not strictly apply. These differences should be understood when one is discussing the clinical interpretation of serologic tests for amebiasis.

Although a large number of people throughout the world are infected with this organism, only a small percentage develop clinical symptoms. Morbidity and mortality due to *E. histolytica* vary depending on the geographic area, organism strain, and patient immune status. Recognition of *Entamoeba dispar*, *Entamoeba moshkovskii*, and *Entamoeba bangladeshi* in humans has further complicated the diagnosis of *E. histolytica*. since these species are morphologically indistinguishable unless ingested red blood cells (RBCs) are found within the trophozoite cytoplasm (true pathogen, *E. histolytica*).

Research emphasis has been on the molecular differences between pathogenic *E. histolytica* and nonpathogenic *E. dispar*. However, the molecules considered the most important for host tissue destruction (amebapore, galactose/*N*-acetylgalactosamine-inhibitable lectin, and cysteine proteases) can be found in both organisms. Pathogenicity differences may be related to the composition and properties of the surface coat components (or pathogen-associated molecular patterns) and the ability of the innate immune response to recognize these components, thus eliminating the organisms. Targets of the host immune system modulation appear to be both neutrophils and macrophages, which are unable to abort the infection even when present at the site of the lesion. The total body of evidence supports the classification of the pathogenic *E. histolytica* and the nonpathogenic *E. dispar* as two distinct species.

Intestinal Disease

In patients with intestinal disease, symptoms range from none to acute or chronic amebic colitis, both of which can mimic inflammatory bowel disease. A number of factors influence the outcome of an amebic infection, including the host immune system response. At one time, it was thought that invasive strains were found only in tropical areas of the world; however, it is now apparent that there are strains within the United States that cause hepatic abscess in patients who have no history of travel outside the United States. Groups at highest risk of having serious sequelae are children, the elderly, the malnourished, and patients receiving corticosteroid therapy. The ascending

TABLE 30.3 Diagnosing parasitic infection in the compromised host[a]

Organism	Size	Specimen	Procedure[b]	Method of examination	Laboratory findings	Comments[c]
Entamoeba histolytica Intestinal disease Note: Pathogenic *E. histolytica* can now be differentiated from nonpathogenic *E. dispar* using immunoassays; morphologic characteristics are identical, *E. histolytica* containing RBCs in the trophozoite could be identified as pathogenic true *E. histolytica*.	Trophozoite: usual range, 15–20 µm; range, 10–60 µm. Cyst: usual range, 12–15 µm; range, 8.5–20 µm. Note: These sizes refer to organisms seen on the permanent stained smear. There may be as much as 1–1.5 µm of shrinkage of organisms as a result of reagents	Stool	O&P examination (minimum of concentration and permanent stained smear) IAs	Concentration (high dry, ×400) IA depends on format	Trophozoites or cysts Presence or absence of antigen	A series of 3 stools (collected every other day) is recommended for the O&P examination Can detect *Entamoeba histolytica* or the *E. histolytica*/*E. dispar* group
		Sigmoidoscopic biopsy or scrapings	Permanent stained smear and routine histologic test	Permanent stain (oil immersion, ×1,000)	Trophozoites	The permanent stained smear is mandatory for a complete O&P examination
		Serum	Amebic serologic tests	IHA, IFA, CIE, ID, ELISA	Not recommended for intestinal disease; negative or possible low titer, indicating present or past exposure	At least 2 different serologic tests (methods) should be performed so results can be compared
				Radiographic studies	Barium	Intestinal abnormalities (may also be normal)
Extraintestinal disease	Trophozoite stage only	Stool	O&P examination	See above	No organisms recovered in about 50% of cases	There may be no evidence of GI disease before or during extraintestinal invasion
		Sigmoidoscopic biopsy or scrapings	Permanent stained smear and routine histologic test	See above	No organisms recovered in about 50% of cases	
		Serum	Amebic serologic tests (antibody); IHA, EIA reagents available	See above	Positive titers may be lower in compromised patients	At least 2 serologic tests (methods) should be performed so that results can be compared
			Scans		Evidence of space-occupying lesion	
Free-living amebae						
Naegleria fowleri	Biphasic (amebic and flagellate forms). Trophozoite: usual range, 8–15 µm, amebic form, lobate pseudopodia. Cyst: not present in tissue, small, smooth, rounded	CSF	Centrifugation, wet or stained preparation; agar plates with bacterial overlay (culture)	Wet: low (×100) and high dry (×400); stained: oil immersion (×500, ×600, or×1,000); culture: low (×100) and high dry (×400) on material from culture plate surface	Trophozoites in specimens; trophozoites and cysts in culture	Since all these organisms are normally free-living, they will survive shipment via mail to any laboratory for subsequent examination and/or culture; various culture temperatures are recommended in different situations

Acanthamoeba spp.	Trophozoite: large, 15–25 µm, amebic form, spiky pseudopodia. Cyst: present in tissue, large with wrinkled double wall.	CSF, eye specimens, cutaneous biopsy	Centrifugation, wet or stained preparation; agar plates with bacterial overlay (culture); histology	As above, histology and routine microscopic exam	Trophozoites and/or cysts in specimens and culture	Agar plates with bacterial overlay (culture) more sensitive than wet preparations
Balamuthia mandrillaris	Trophozoite: large, 12–60 µm, amebic form, fingerlike pseudopodia. Cyst: large, 6–30 µm, with wrinkled double wall.	CSF, eye specimens, cutaneous biopsy	Centrifugation, wet or stained preparation; cell culture only, will not grow on routine agar culture with bacterial overlay; histology	All of the above, including cell culture if available	Trophozoites and/or cysts in specimens and cell culture	Becoming more widely recognized as causing same types of disease as *Acanthamoeba* spp.
Sappinia sp.	Trophozoite: 40–80 µm, oblong or ovoid; flattened with wrinkles on the surface. Cyst: 15–30 µm.	CSF, biopsy, MRI, PCR	Centrifugation, wet or stained preparation; agar plates with bacterial overlay (culture); histology	As above, histology and routine microscopic exam	Trophozoites and/or cysts in specimens and culture	Can be distinguished from other amebae by the presence of a distinctive double nucleus in which the 2 nuclei are closely apposed with a central flattening; 2 nucleoli
Blastocystis spp.	Vacuolar forms usual range, 3–120 µm. Human (5–15 µm)	Stool	O&P examination (minimum of concentration and permanent stained smear)	Concentration (high dry, ×400); permanent stain (oil immersion, ×1,000)	Central body form (vacuolar); other forms may be present, but they are difficult to identify	Central vacuole fills 70–90% of cell; giant cells with multiple nuclei occasionally seen; cytoplasm and organelles limited to thin peripheral rim (may be difficult to see in wet mounts); most common form seen in microscopy of fecal specimens
Giardia lamblia	Trophozoite: usual range, 10–12 µm long by 5–10 µm wide. Cyst: usual range, 11–14 µm long by 7–10 µm wide	Stool	O&P examination Immunoassays	Concentration (high dry, ×400); permanent stain (oil immersion, ×1,000) Depends on format	Trophozoites or cysts (may not always be detected) Presence or absence of antigen or actual organisms	The parasite may not be recovered from stool even after 4 or 5 examinations on different specimens. This does not mean that the diagnostic techniques are inadequate or that the organisms are missed by inexperienced personnel. Additional diagnostic techniques (including biopsy) may have to be used. IAs available and now being used as orderable tests (FA, EIA, rapid cartridge formats).
		Duodenal aspirate	Wet preparations, permanent stained smears	See above	Trophozoites	

(continued)

TABLE 30.3 Diagnosing parasitic infection in the compromised host[a] *(continued)*

Organism	Size	Specimen	Procedure[b]	Method of examination	Laboratory findings	Comments[c]
		Entero-Test capsule	Wet preparations, permanent stained smears	See above	Trophozoites	
		Biopsy	Routine histologic tests and special stains		Possible upper GI tract changes and/or trophozoites	
		Serum	Serologic tests	Experimental (not generally available)		
Toxoplasma gondii	Tachyzoite: 4–6 µm long by 2–3 µm wide. Bradyzoite: individual tissue cysts may contain many hundreds of organisms. Cyst: may range up to 50–75 µm.	Serum	Serologies	IHA, IFA, ELISA, Sabin-Feldman dye test	Results are often sufficient to confirm diagnosis	Patients with positive IgM serologic test results may have Epstein-Barr virus infections. There may be nonspecific stimulation of other antibody-producing cells. Although organism isolation techniques with animals and/or tissue culture may be used, these results must be evaluated within the context of the total clinical picture.
		Biopsy	Routine histologic test		Possible recovery of bradyzoites	Organism may not be etiologic agent of disease
			Tissue culture or animal isolation		Possible recovery of tachyzoites	Organism may not be etiologic agent of disease
		Spinal fluid	Organism isolation (direct, tissue culture, or animal isolation)		Presence of tachyzoites	Considered confirmatory
			Serologic tests		Confirmatory if higher in CSF than in serum	Rare finding
Cryptosporidium spp.	Developmental stages within brush border of intestine. Cells: 1–2 µm. Oocysts in stool: 4–6 µm	Stool	Acid-fast stains (may need modified KOH digestions)	Permanent stain (high dry, ×400; confirm on oil immersion ×1,000)	Oocysts visible	Regardless of staining technique used on stool, the more normal the stool specimen (formed), the greater the chances that artifacts will present problems in identifying organisms and fewer organisms will be present. Specimens representing classic diarrhea will allow easier recovery and ID of organisms. Numbers of organisms will vary from day to day.

			IAs	Depends on format	Presence or absence of antigen or actual oocysts	ELISA, FA, or rapid cartridge format
		Intestinal mucosal biopsy	Routine histology	H&E stain	Developmental stages (1 μm) along brush border, other organs	
			Electron microscopy	Routine EM	May see developmental stages or possibly oocysts	Very specific, but not very sensitive
		Serologies		Experimental only (not generally available)		Used for epidemiologic studies
Cyclospora cayetanensis	Oocyst: 8–10 μm	Stool	Acid-fast stains (modified)	Permanent stain (high dry, ×400; confirm on oil immersion, ×1,000)	Oocysts visible	Owing to morphologic similarities to *Cryptosporidium* spp., it is important to measure the oocysts. There is tremendous staining variability of the oocysts with acid-fast stains (from clear to deep purple). Oocysts appear wrinkled. Oocysts in stool are unsporulated and do not contain any internal definition or structure.
			Wet preparation examinations; oocysts will autofluoresce	Green with 450–490 DM excitation filter, blue with 365 DM excitation filter. Wet preparation (high dry, ×400).	Oocysts visible as green or blue circles (interior of oocyst does not fluoresce) (1+ to 3+)	Used as a screening method
		Intestinal mucosal biopsy	Routine histologic test	H&E stain	Organisms resembling coccidia found in jejunal enterocytes	Developmental location will differentiate from *Cryptosporidium* spp.
			EM	Routine EM	May see developmental stages or possibly oocysts	

(continued)

TABLE 30.3 Diagnosing parasitic infection in the compromised host[a] *(continued)*

Organism	Size	Specimen	Procedure[b]	Method of examination	Laboratory findings	Comments[c]
Cystoisospora (*Isospora*) *belli*	Oocyst: 30 by 20 µm, development within the intestinal mucosal cells; oocysts in stool range from undivided mass of protoplasm to those containing fully developed sporocysts and sporozoites	Stool	O&P examination (concentration is important; PVA distorts morphology)	Concentration (low, ×100, and high dry, ×400)	Oocysts (may be in various stages of development)	Although these organisms are thought to be rare, some workers speculate that oocysts are occasionally missed during a stool examination. There is also the possibility that a biopsy specimen is positive while the stool examination is negative.
			Permanent stained smear	Acid-fast stains	Oocysts (as above). High dry, ×400.	
		Intestinal mucosal biopsy	Routine histologic test	H&E stain, high dry (×400)	Developmental stages within the mucosal cells	
Sarcocystis suihominis, *S. bovihominis*	Oocyst thin walled, contains 2 mature sporocysts, each with 4 sporozoites; sporocysts are 9–16 µm long by 7.5–12 µm wide	Stool	O&P examination (concentration important; PVA may distort morphology)	Concentration (low, ×100, and high dry, ×400)	Oocysts or sporocysts	Not commonly seen; size can overlap that of *C. belli*
Other *Sarcocystis* spp.	Shapes and sizes of skeletal and cardiac muscle sarcocysts vary	Tissue biopsy	Routine histology	H&E stain (high dry, ×400)	Developmental stages within tissue cells	Sarcocysts contain several hundred to thousands of trophozoites, each measuring 4–9 µm wide by 12–16 µm long; sarcocysts may be divided into compartments by septa (not seen in *Toxoplasma* cysts)
Microsporidia[d] (*Anncaliia*, *Encephalitozoon*, *Enterocytozoon*, *Pleistophora*, *Trachipleistophora*, *Microsporidium* spp., *Vittaforma*, *Tubulinosema*)	Spore, 1–4 µm; dividing forms within epithelial cells	Tissue biopsy	Routine histologic test	Methenamine silver, acid-fast, PAS-positive granule, Giemsa, H&E	Spores, dividing forms	It is sometimes difficult to find and identify the organisms by routine histologic techniques. Because of the difficulties in identification at the light microscopy level, EM methods have been recommended. Organisms have been identified in stool, although current procedures may be somewhat difficult to interpret
			EM	Routine EM	Spores, dividing forms	Specific but insensitive
		Stool	Permanent stained smear	Modified trichrome	Spores	Artifacts confusing
			EM Direct detection methods	Routine EM IFA; not yet available commercially	Spores	Specific but insensitive

		Serologic sample	IFA, CF, microagglutination	Experimental (not generally available)	Titers	
Leishmania spp.	Amastigotes in tissue measure 1–3 µm; tend to appear larger when released from cells, such as a touch or squash preparation; will appear as dots within the cells in routine histology preparations	Blood specimen (looking at buffy coat cells); tissue biopsy (bone marrow most common)	Buffy coat preparations, centrifugation, smear preparation; smears, culture (Novy-MacNeal-Nicolle medium) of bone marrow aspirates	Microscopic examination (high dry, ×400 power and ×1,000 oil immersion)	Presence of amastigotes in macrophages and/or tissues; presence of promastigotes (motile, flagellated) in culture	Multiple specimens may be required; cultures must not become contaminated or results may represent a false negative; controls must accompany all patient cultures
Strongyloides stercoralis	Larval stage: 180–380 by 14–20 µm, in feces; larvae (rhabditiform) have a short buccal cavity and a prominent genital primordium; both noninfective and infective larvae, adults, and eggs may be found in duodenal specimens	Stool	O&P examination (concentration important)	Direct smears and concentration (low, ×100; confirm on high dry, ×400)	Rhabditiform larvae (infective filariform larvae may also be found)	Caution should be used when working with fresh specimens that may contain infective filariform larvae. *Strongyloides* infections may be difficult to diagnose from stool specimens. Examination of material from the duodenum may be necessary, and concentrations or cultures can also be used.
		Duodenal aspirate	Baermann concentrate, Harada-Mori and petri dish cultures	As above	As above	
			Wet preparations (direct smears)	As above	Larvae (usually rhabditiform)	
		Entero-Test	Wet preparations (direct smears)	As above	Larvae (as above)	
Plasmodium spp.	Young rings may be missed without use of the 100× oil immersion objective	Blood	Thick and thin stained blood films	1,000× objective for minimum of 300 fields, each blood film	Various stages of the life cycle	Parasitemias may be higher than normal in HIV coinfections; do not tend to see rings outside of the RBCs
Babesia spp.	Ring forms may be missed without use of the 100× oil immersion objective; may mimic *P. falciparum* ring forms	Blood	Thick and thin stained blood films	100× objective for minimum of 300 fields, each blood film	Ring forms, may or may not see Maltese cross configuration	Parasitemias may be higher than those seen in malaria infections; may see rings outside of the RBCs
Trypanosoma cruzi	Trypomastigotes require 40× or 100× objectives	Blood, CSF	Thick and thin stained blood films; buffy coat films	100× objective for minimum of 300 fields, each blood film	Trypomastigotes	May also find organisms in histologic preparations (cardiac myopathy, GI tract myopathy)

(continued)

TABLE 30.3 Diagnosing parasitic infection in the compromised host[a] *(continued)*

Organism	Size	Specimen	Procedure[b]	Method of examination	Laboratory findings	Comments[c]
Crusted scabies (*Sarcoptes scabiei*)	Mites are microscopic; live in cutaneous burrows where female deposits eggs; mites range from 215 to 390 µm in length; eggs are 170 µm long by 92 µm wide; fecal pellets are yellow-brown	Skin scrapings	Use skin-scraping technique (see chapter 9)	Prepare slides of skin-scraping material and examine microscopically using low power (×100) and high power (×400)	Mites, eggs, or scybala (fecal pellets) may be present	Highly infectious; most common skin sites are interdigital spaces, backs of the hands, elbows, axillae, groin, breasts, umbilicus, penis, shoulder blades, small of the back, buttocks; may see kerotic excrescences on the body; itching may be absent.

[a] CF, complement fixation; CIE, counterimmunoelectrophoresis; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; EM, electron microscopy; FA, fluorescence assay; GI, gastrointestinal; H&E, hematoxylin and eosin; IA, immunoassay; ID, immunodiffusion; IFA, immunofluorescent assay; IHA, indirect hemagglutination assay; MRI, magnetic resonance imagery; O&P, ova and parasite; PVA, polyvinyl alcohol; RBCs, red blood cells.

[b] Some parasites (*Giardia, Entamoeba histolytica, Cryptosporidium, Cyclospora*) are now being detected with molecular PCR panels (automated); additional organism targets are currently being developed.

[c] The use of immunoassays for the direct and indirect detection of some of these parasites has become much more common. Reagents are available for *Entamoeba histolytica/E. dispar*, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium* spp., and the microsporidia (not currently available within the United States).

[d] This genus has been reported in humans; organisms that have not yet been classified to genus have been placed in the genus *Microsporidium*, which has now been accepted as a genus name rather than a "catchall" term.

colon is the most affected region of the large intestine. Severe colitis presents with severe dysentery, abdominal pain, and, rarely, fever. The extensive necrotizing colitis is often fatal.

The diagnosis of amebiasis begins with an ova and parasite examination. This procedure includes the following: the direct saline mount, which is designed to allow the mobility of the organisms to be seen; the concentration procedure, which provides a method to recover helminth eggs and larvae and protozoan cysts; and the permanent stained smear, which is the most important technique for diagnosis of the intestinal protozoa. Although motility can be seen on direct wet preparations, the material must be fresh, and a diagnosis of amebic infection should *never* be made solely from this type of examination. At the very least, the concentration procedure and permanent stained smear should be performed on every stool sample that is submitted to the laboratory for an ova and parasite examination (1). Many clinical laboratories now receive stool submitted in fecal fixatives; thus, the ova and parasite examination would consist of the concentration and permanent stained smear. This eliminates the lag time between stool passage and fixation, thus removing the problem of organism disintegration and false-negative reports.

Stools should normally be submitted to the laboratory on an every-other-day basis for a period of no more than 10 days. Although for many years the recommended minimum number of stools to be examined has been three, some laboratories are accepting two specimens. When intestinal amebiasis is suspected, six specimens may be submitted over a 15-day period. Multiple specimens are recommended because populations of intestinal protozoa tend to be cyclic. That is, the specimen may be negative on collection day 1 but positive by collection day 3. It is also possible for only a nonpathogen to be found on collection day 1 but pathogenic organisms to be recovered from the second or third specimen. If nonpathogenic protozoa are found, there is always the possibility of finding pathogenic organisms on subsequent examinations.

Presumptive identification of the organism as *Entamoeba histolytica/E. dispar* is based on the typical nuclear morphology (evenly arranged chromatin on the membrane and the presence of a small, central karyosome). The presence of RBCs within the trophozoite cytoplasm would provide definitive identification as true *E. histolytica* (rather than nonpathogenic *E. dispar*); however, RBCs must also be present in the background of the smear if they are reported to be inside the cytoplasm of the organism. In general, if the patient is symptomatic, one assumes that the causative agent is pathogenic *E. histolytica* (assuming that the cause is related to the presence of intestinal protozoa).

TABLE 30.4 Parasitic infections: clinical findings in normal and compromised hosts

Organism	Normal host	Compromised host
Entamoeba histolytica	Asymptomatic to chronic or acute colitis; extraintestinal disease may also occur (primary site: right upper lobe of liver)	Diminished immune system capacity may lead to extraintestinal disease
Free-living amebae	Primary amebic meningoencephalitis (PAM); patients tend to have eye infections with *Acanthamoeba* spp.; linked to poor lens care	PAM; granulomatous amebic encephalitis (GAE)
Blastocystis spp.	Asymptomatic to diarrhea; most common protozoan found in gastrointestinal tract throughout the world	Has been associated with infections in immunocompromised patients; 9/13 subtypes seen in humans; some pathogenic, some not; implicated in extraintestinal disease, as well
Giardia lamblia	Asymptomatic to malabsorption syndrome	Certain immunodeficiencies tend to predispose an individual to infection
Toxoplasma gondii	Approximately 50% of individuals have antibody and organisms in tissue but are asymptomatic	Disease in compromised host tends to involve the central nervous system with various neurological symptoms; *Toxoplasma* encephalitis (TE)
Cryptosporidium spp.	Self-limiting infection with diarrhea and abdominal pain	Owing to autoinfective nature of life cycle, is not self-limiting, may produce fluid loss of over 10 liters/day, and there may be multisystem involvement; no known totally effective therapy
Cyclospora cayetanensis	Self-limiting infection with diarrhea (3–4 days), with relapses common	Diarrhea may persist for 12 wk or more; biliary disease has also been reported in this group, particularly in those with AIDS
Cystoisospora (*Isospora*) *belli*	Self-limiting infection with mild diarrhea or no symptoms	May lead to severe diarrhea, abdominal pain, and possible death (rare case reports); diagnosis is occasionally missed because of nonrecognition of the oocyst stage; is not seen when concentrated from polyvinyl alcohol fixative
Sarcocystis spp.	Self-limiting infection with diarrhea, or mild symptoms	Symptoms may be more severe and last longer
Microsporidia (*Anncaliia*, *Encephalitozoon*, *Enterocytozoon*, *Pleistophora*, *Trachipleistophora*, *Tubulinosema*, *Microsporidium*)	Little known about these infections in the normal host; serologic evidence suggests infections may be more common than recognized	Can infect various parts of the body; diagnosis often depends on histologic examination of tissues; routine examination of clinical specimens (stool, urine, etc.) becoming more common; can probably cause death
Leishmania spp.	Asymptomatic to mild disease	More serious manifestations of visceral leishmaniasis; some cutaneous species will manifest visceral disease; difficult to treat and manage; definite coinfection with AIDS
Strongyloides stercoralis	Asymptomatic to mild abdominal complaints; can remain latent for many years owing to low-level infection maintained by internal autoinfective life cycle	Can result in disseminated disease (hyperinfection syndrome due to autoinfective nature of life cycle); abdominal pain, pneumonitis, sepsis-meningitis with Gram-negative bacilli, eosinophilia; distinct link to some leukemias and lymphomas; can be fatal
Plasmodium spp.	Asymptomatic to severe symptoms, depending on immune status of patient	Parasitemias tend to be higher, particularly with HIV coinfections
Babesia spp.	Asymptomatic, self-cure in immunocompetent patient (*B. microti*); may be more severe in patients with *B. divergens* or *B. duncani*	May cause severe symptoms, even death, in patients who have been splenectomized or who are immunocompromised for other reasons
Trypanosoma cruzi, American trypanosomiasis	In children younger than 5 years, the disease is seen in its severest form, whereas in older children and adults, the disease is milder and is commonly diagnosed in the subacute or chronic form rather than in the acute form; acute-stage symptoms only occur in about 1% of patients; recovery may occur without therapy	Individuals who were previously infected with *T. cruzi* and later become positive for HIV are at risk of reactivation of their Chagas' disease; these patients develop a severe multifocal or diffuse meningoencephalitis with numerous tissue parasites; associated with high mortality
Crusted scabies (*Sarcoptes scabiei*)	Infections can range from asymptomatic to moderate itching	Severe infection with reduced itching response; hundreds or thousands of mites on body; infection very easily transferred to others; secondary infection very common

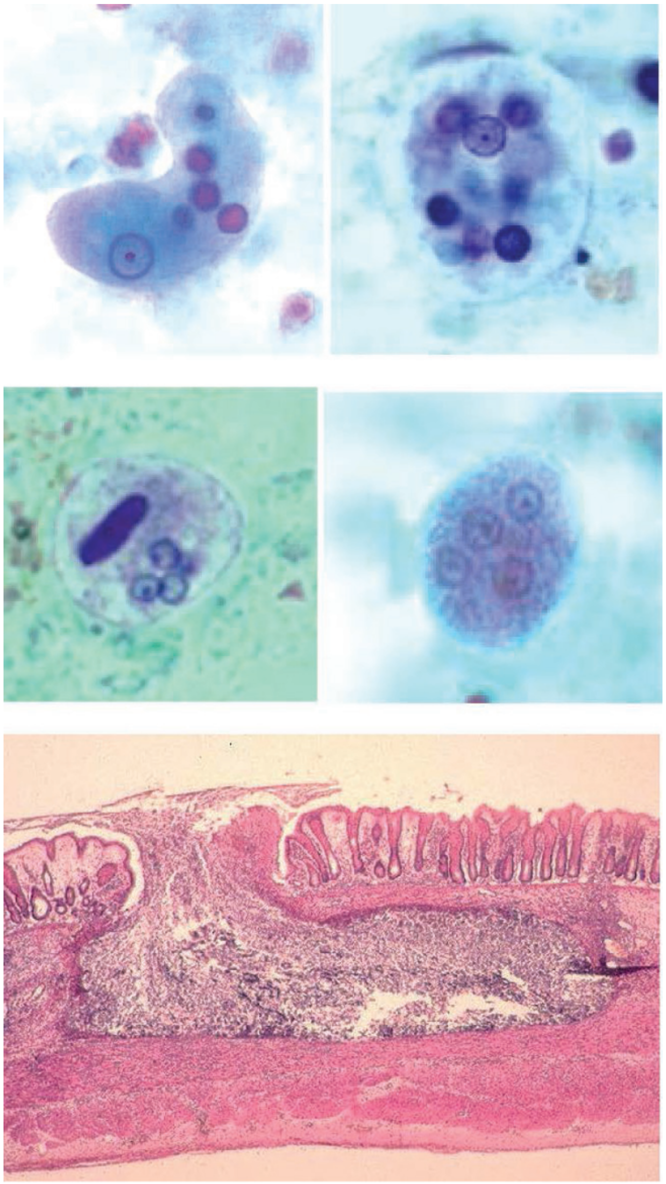


Figure 30.1 (Top) *Entamoeba histolytica* trophozoites containing ingested RBCs. (Middle) *Entamoeba histolytica/E. dispar* cysts. (Bottom) Amebic liver abscess in section; note the "flask-shaped" ulcer. doi:10.1128/9781555819002.ch30.f1

The cyst of *E. histolytica/E. dispar* may contain chromatoidal bars with smooth, rounded edges. Even though the mature cyst contains four nuclei, these are not always visible, and the cyst can often be identified from the chromatoidal bars alone.

In a wet preparation, such as the direct smear or concentration sediment, the trophozoites and cysts of *E. histolytica* measure >12 and >10 µm, respectively. Because of artificial shrinkage during the preparation of permanent stained smears, the sizes are diminished somewhat, with the trophozoites measuring at least 10.5 to 11 µm and the cysts measuring at least 8.5 to 9 µm. Organisms measuring less than these limits and containing morphologic characteristics similar to those of *E. histolytica* would be identified as *E. hartmanni*, a nonpathogenic ameba.

One of the most important things to remember is that some human cells found in the stool can mimic *E. histolytica*. Macrophages or monocytes can look like the trophozoite form of *E. histolytica*, and polymorphonuclear leukocytes, when they have been in the stool for a while, can mimic the four-nucleus *E. histolytica* mature cyst. However, if the patient has diarrhea or dysentery, it is very unusual to find cysts in the stool; the gut motility is so rapid, there is not time for cyst formation.

Other tests include sigmoidoscopy, in which both scrapings and smears can be submitted for permanent stains. Biopsy specimens should also be submitted for histopathologic studies. Radiographic examination with barium is helpful; however, the presence of barium in the stool makes the ova and parasite examination very difficult to perform. Ova and parasite examinations should be done before barium studies or at least 1 week to 10 days afterward. The sensitivity of serologic tests for antibody in patients with intestinal amebiasis varies, and a negative serologic test does not necessarily rule out the presence of amebic infection. Serologic tests for antibody are not recommended for the diagnosis of intestinal amebiasis.

Tests for antigen detection in the stool are much more relevant for intestinal disease. Reagents are currently available for antigen detection of the *E. histolytica/E. dispar* group and specifically for *E. histolytica*. Fresh, frozen, or stool submitted in Cary-Blair medium is required for the procedure (confirm with the package insert); PCR, isoenzyme analysis, and antigen detection are available for confirmation of *E. histolytica* infection. However, the antigen detection method is both rapid and technically simple.

If *E. histolytica* organisms are not seen in the stool and the final diagnosis is inflammatory bowel disease, the patient may receive corticosteroid therapy. Immunosuppression may predispose the patient to more severe disease if the true pathogen, *E. histolytica*, is present and not detected.

Extraintestinal Disease

When organisms invade the mucosal lining and are carried via the bloodstream to the liver via the portal vein (extraintestinal or hepatic disease), a somewhat different approach to diagnosis is necessary. Both fecal and sigmoidoscopic examinations for organisms are negative in approximately half of the patients with extraintestinal amebiasis. Both radioactive and computed tomographic (CT) scans are available to assist the clinician in defining the presence of an abscess.

Lesions in the liver may range from less than a few centimeters to several inches in diameter. In either case, if the patient has a normal humoral immune system, serologic tests for antibody are generally positive.

Serologic tests for extraintestinal amebiasis are generally very specific and quite sensitive. However, in some parts of the country, sensitivity may present a problem in patients previously exposed to *E. histolytica*. In these individuals, a low to moderate titer may represent past infection rather than current disease. Also, patients with diminished immunoglobulin levels may present with low or negative serologic titers (Table 30.3). Various serologic techniques include complement fixation, indirect hemagglutination assay (IHA), counterimmunoelectrophoresis, latex agglutination, indirect fluorescent-antibody (IFA) assay, and enzyme-linked immunosorbent assay (ELISA), as well as tests for the detection of antigen in the stool. It is generally recommended that two different procedures be used so that the results can be compared. One approach is to use both qualitative screen and quantitative titer procedures. Commercial suppliers of kits are limited, and few laboratories routinely provide this type of testing. Serum for serologic tests should be sent to the state and local public health laboratories if the test is not routinely available.

AIDS Patients. Invasive amebiasis appears to be an emerging parasitic disease in patients infected with HIV in areas where amebic infection is endemic. Medical, microbiological, and histopathologic records of 296 HIV-infected patients and serologic data from IHAs of samples from 126 HIV-infected patients were reviewed to identify cases of invasive amebiasis. An IHA titer of 1:128 was considered positive. Of these 296 HIV-infected patients, 18 (6.1%) were diagnosed with invasive amebiasis. Clinical manifestations included amebic colitis (13 patients), amebic liver abscess (9 patients), both colitis and abscess (4 patients), and pleural effusion (2 patients). Invasive amebiasis was the initial presentation of HIV infection in nine patients. Of the 18 patients diagnosed with invasive amebiasis, 13 had an IHA titer of ≥1:128. The sensitivity of the IHA in the diagnosis of invasive amebiasis was 72.2%, and the specificity was 99.1%. The positive predictive value of IHA for invasive amebiasis in this patient population was 92.9% and the negative predictive value was 95.5% (2). It appears that invasive amebiasis is becoming more important as one of the opportunistic parasitic infections in patients with HIV infection in areas of amebiasis endemicity. In this type of area of endemicity, the IHA should be considered a relevant diagnostic approach.

In a study from Mexico, the prevalence estimated with PCR data showed that *E. histolytica* infection was more common in the HIV-positive/AIDS group (25.32%) than in HIV-negative contacts (18.46%). *E. histolytica/E. dispar* infection was more frequent in HIV-positive/AIDS patients (13.3%) than in HIV-negative contacts (0.7%). However, *E. histolytica* and/or *E. histolytica/E. dispar* infection was highly prevalent in HIV-positive/AIDS patients (34.1%) without evidence of recent or current invasive disease. Contacts of HIV-positive/AIDS patients who were infected with *E. histolytica/E. dispar* were asymptomatic cyst passers. These data indicate that *E. histolytica* strains prevalent in the studied community appear to be of low pathogenic potential within the compromised patient, and infection with HIV-1 does not appear to be a risk factor for amebiasis (3).

However, in another study, 49 (5.2%) of 951 HIV-infected persons had 51 episodes of invasive amebiasis. A high IHA titer was detected in 39 (6.2%) of 634 HIV-infected persons compared with 10 (2.3%) of 429 uninfected controls with gastrointestinal symptoms and 0 of 178 uninfected healthy controls. Stool specimens from 40 (12.1%) of 332 HIV-infected persons and 2 (1.4%) of 144 uninfected healthy controls were positive for *E. histolytica* or *E. dispar* antigen. Of the 40 antigen-positive stool specimens from HIV-infected persons, 10 (25.0%) contained *E. histolytica*. Therefore, persons infected with HIV in Taiwan are at increased risk for invasive amebiasis and exhibit a relatively high frequency of elevated antibody titers and intestinal colonization with *E. histolytica* (2).

High-risk areas for acquiring amebiasis include Mexico, the western portion of South America, western Africa, South Africa, parts of the Middle East, and South and Southeast Asia. Invasive disease is much more common in these areas of the world.

Free-Living Amebae

Small, free-living amebae are becoming recognized clinically as important parasitic pathogens causing infections, particularly in immunocompromised patients (Table 30.5) (4). Primary amebic meningoencephalitis (PAM) caused by *Naegleria fowleri* and granulomatous amebic encephalitis (GAE) caused by *Acanthamoeba* spp. and *Balamuthia mandrillaris* (including cases in patients with AIDS) are now well recognized (see Fig. 30.2 and 30.3). Another identified group of infections caused by these organisms is *Acanthamoeba* keratitis, which is related primarily to poor lens care in those who wear contact lenses. When free-living, these organisms feed on bacteria and nutrients in moist soil and in fresh and marine waters.

Although it is well known that both free-living and pathogenic protozoa harbor a variety of endosymbiotic bacteria, it is unclear what role these organisms play in

TABLE 30.5 Comparison of diseases caused by the most common free-living amebae

Determinant	*Naegleria fowleri*	*Acanthamoeba* sp., *Balamuthia mandrillaris*
Stages	Trophozoites: 10–15 µm, with broad, rounded pseudopods. Cyst: 7–10 µm, round, thick double wall	Trophozoites: 25–40 µm with slender, pointed/spiky pseudopods. Cyst hexagonal with double wall, spherical
Disease	Primary amebic meningoencephalitis (PAM)	Granulomatous amebic encephalitis (GAE); *Acanthamoeba* keratitis
Epidemiology	Good health, recent history of swimming in lake or other warm water natural sources, including nontreated swimming pools; use of neti pots for nasal irrigation with contaminated water; also inhalation via dust	Immunocompromised (e.g., AIDS); other causes such as various diseases and/or transplantation
Predisposing conditions	None	Alcoholism, pregnancy, systemic lupus erythematosus, hematologic disorders, AIDS, chemotherapy, radiation therapy, transplantation, and steroid treatment
Incubation	3–7 days	Probably >10 days
Portal of entry	Olfactory neuroepithelium	Skin, lungs, olfactory neuroepithelium
Onset	Very rapid	Slow, insidious
CNS spread	Direct	Probably hematogenous
Organs affected	Brain only	Brain, skin, eyes, lungs
Clinical course	Acute, fulminant; fatal within 10 days or less	Subacute (8–30 days) or chronic (>32 days)
Signs and symptoms	Severe headache, anorexia, nausea, vomiting, fever, mental abnormalities, stuffy/blocked nose	Mental abnormalities, seizures, fever, hemiparesis, headache, visual abnormalities
Ocular involvement	None	Keratitis
CSF	Similar to bacterial meningitis (clear to purulent); neutrophilic pleocytosis, high protein, low glucose	CSF similar to those in viral encephalitides
Laboratory diagnosis	Direct examination of fresh CSF shows active trophozoites (recommended: slide with coverslip, not counting chamber); culture, nonnutrient agar plate with bacterial overlay; calcofluor white for cysts	Culture, nonnutrient agar plate with bacterial overlay; calcofluor white for cysts
Potential diagnostic problems	Bacterial meningitis diagnosed on the basis of false-positive Gram stain for bacteria; antibiotics not effective for PAM; false-positive Gram stain, BUT negative routine bacterial cultures; amebae in CSF in counting chamber may be confused with PMNs and/or monocytes	Specimens diluted on too many agar plates (temperature options), resulting in a false-negative culture; amebae in CSF in counting chamber may be confused with PMNs and/or monocytes.
Host response	Purulent leptomeningitis, hemorrhagic necrotizing meningoencephalitis, brain edema, perivascular collection of amebas	Granulomatous encephalitis with focal necrosis and multinucleated giant cells, necrotizing angitis
CNS amebic forms	Trophozoites	Trophozoites and cysts
Differential diagnosis	Acute pyogenic (bacterial) leptomeningitis	Tuberculous, viral, or fungal encephalitis; brain tumors, brain abscess; ocular herpes; fungal keratitis
Therapy	Amphotericin B + miconazole + rifampin	A topical biguanide, 0.02% chlorhexidine or polyhexamethylene biguanide (PHMB, 0.02%), either alone or combined with a diamidine, propamidine isethionate (Brolene) or hexamidine (Desomodine)

terms of host survival, infectivity, and invasiveness. *Legionella pneumophila* can infect, multiply within, and kill both *Naegleria* and *Acanthamoeba* amebae. These amebae may be natural hosts for *Legionella*, as well as for other bacteria such as *Listeria monocytogenes*, *Vibrio cholerae*, *Mycobacterium leprae*, and *Pseudomonas aeruginosa*. Because of their well-known resistance to chlorine, the amebic cysts are considered to be vectors for these intracellular bacteria. This can have tremendous significance for any hospital where the water source is contaminated with free-living amebae. There is also evidence to suggest that the presence of amebae in domestic water supplies may provide growth conditions that enhance the pathogenicity of the organism for the human host. These amebae are also acceptable hosts for echoviruses and polioviruses. The epidemiology, immunology, protozoology, pathology, and clinical features of the infections produced by these free-living protozoa differ considerably.

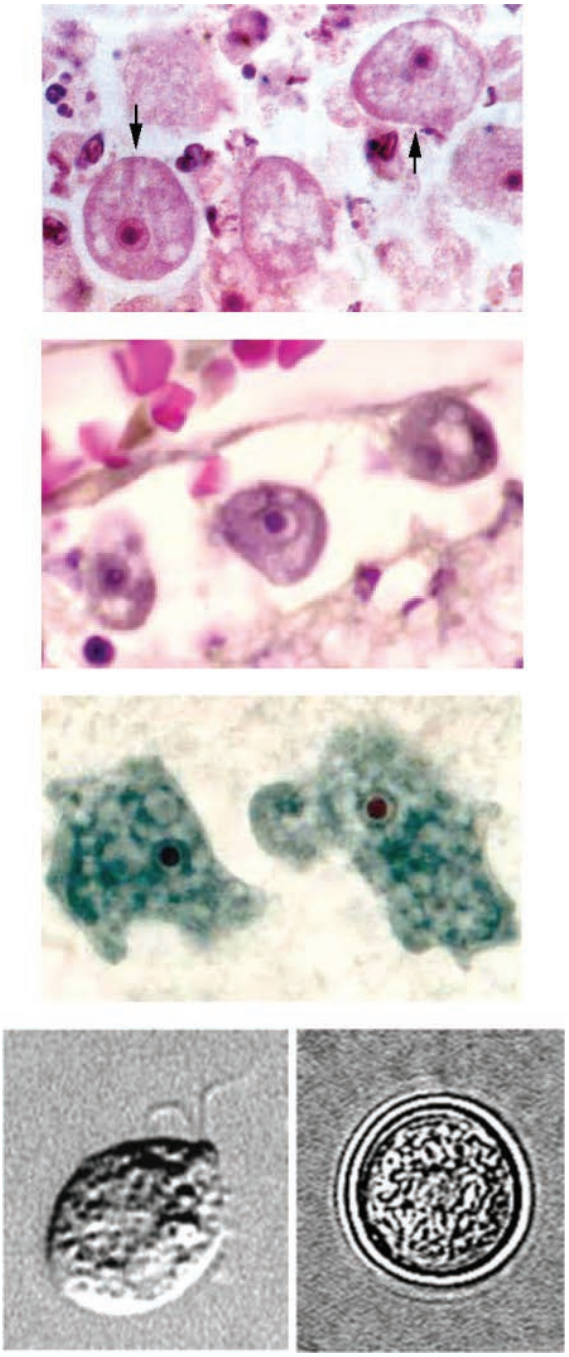


Figure 30.2 (Top) *Naegleria fowleri* trophozoite (note the large karyosome within the nucleus). (Row 2) *N. fowleri* trophozoites within brain tissue. (Row 3) *N. fowleri* trophozoites; note "globby/rounded" pseudopods. (Bottom, left) Flagellated form of *Naegleria*; (right) cyst of *Naegleria*, not seen in human tissue. (Bottom images courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch30.f2

Naegleria fowleri

Central nervous system (CNS) infections caused by free-living amebae have been recognized only since the mid-1960s. *N. fowleri* causes PAM, a fulminant and rapidly fatal disease that affects mainly children and young adults (Fig. 30.2). The disease closely resembles bacterial meningitis but is caused by this free-living ameba, an organism found in moist soil and freshwater habitats. Although PAM is usually associated with healthy, young individuals with a history of recent water-related sport activities, it is an important infection to consider in both immunocompetent and immunocompromised individuals. However, almost all infections have been seen in otherwise healthy individuals. Close to 200 cases of PAM have occurred worldwide, and approximately 90 of those cases have been reported from the United States.

Life Cycle. There are both trophozoite and cyst stages in the life cycle, and the stage depends primarily on environmental conditions. Trophozoites can be found in water or moist soil and can be maintained in tissue culture or other artificial media. The trophozoites can occur in two forms, ameboid and flagellate. Motility can be observed in hanging-drop preparations from cultures of cerebrospinal fluid (CSF); the ameboid form (the only form recognized in humans) is elongate with a broad anterior end and a tapered posterior end. The size ranges from 7 to 20 µm. The diameter of the rounded forms is usually 15 µm. There is a large central karyosome and no peripheral nuclear chromatin. The cytoplasm is somewhat granular and contains vacuoles. The ameboid-form organisms change to the flagellate form when they are transferred from culture or teased from tissue into water and maintained at a temperature of 27 to 37°C. The flagellate form is pear shaped, with two flagella at the broad end. Motility is typical, with either spinning or jerky movements. These flagellate forms do not divide, but when the flagella are lost, the ameboid forms resume reproduction. Cysts from nature and from agar cultures look the same and have a single nucleus almost identical to that seen in the trophozoite. The cysts are generally round, measuring 7 to 10 µm in diameter, with a thick double wall.

Amebic Meningoencephalitis. PAM caused by *N. fowleri* is an acute, suppurative infection of the brain and meninges. In humans, with extremely rare exceptions, the disease is rapidly fatal. The period between contact with the organism and onset of clinical symptoms such as fever, headache, and rhinitis may vary from 2 to 3 days to as long as 7 to 15 days.

The amebae may enter the nasal cavity by inhalation or aspiration of water, dust, or aerosols containing the trophozoites or cysts. The organisms then penetrate the nasal mucosa, probably through phagocytosis of the olfactory epithelial cells, and migrate via the olfactory nerves to the brain. Early symptoms include vague upper respiratory distress, headache, lethargy, and occasionally olfactory problems. Cysts of *N. fowleri* are generally not seen in brain tissue. The acute phase includes sore throat; stuffy, blocked, or discharging nose; and severe headache. Progressive symptoms include pyrexia, vomiting, and stiffness of the neck. Mental confusion and coma usually occur approximately 3 to 5 days prior to death. The cause of death is usually cardiorespiratory arrest and pulmonary edema.

Organ Transplantation, Other Immunocompromised Conditions. The first case of organ transplantation from a donor who had died of undiagnosed *N. fowleri* infection was reported in 1997 (5, 6). While no subsequent amebic infections occurred in the three organ recipients, this report emphasizes the need for adequate evaluation of the benefits and risks of transplanting tissues from persons whose illness might have been caused by an infectious agent. Apparently, the Centers for Disease Control and Prevention (CDC) is also aware of another instance in which kidneys were transplanted prior to determination that the donor was infected with *N. fowleri* (5, 6). At present, the risk of transmission of *N. fowleri* by donor organs has not been clarified, and no practical test is available to ensure that donor organs are organism free. Also, no prophylactic drug regimen to treat transplant recipients has been established.

Another case was reported in a patient with systemic lupus erythematosus. Although treatment was undertaken with intravenous amphotericin B, the patient suffered a fatal relapse while still on therapy (7).

Diagnosis. PAM can resemble acute purulent bacterial meningitis and may be difficult to differentiate, particularly in the early stages. The CSF may have the predominantly polymorphonuclear leukocytosis, increased protein concentration, and decreased glucose concentration that are seen with bacterial meningitis. Unfortunately, if the CSF Gram stain is interpreted incorrectly (identification of bacteria as a false-positive result), the antibacterial therapy will have no effect on the amebae and the patient will usually die within several days. Extensive tissue damage occurs along the path of amebic invasion; the nasopharyngeal mucosa shows ulceration, and the olfactory nerves are inflamed and necrotic. Hemorrhagic necrosis is concentrated in the region of the olfactory bulbs and the base of the brain. Organisms can be found in the meninges, perivascular spaces, and sanguinopurulent exudates.

Clinical and laboratory data usually cannot be used to differentiate pyogenic meningitis from PAM, and so the diagnosis may have to be reached by a process of elimination. A high index of suspicion is often mandatory for early diagnosis. Although most cases are associated with exposure to contaminated water through swimming or bathing, this is not always the case. The rapidly fatal course of 4 to 6 days after the beginning of symptoms (with an incubation period of 1 day to 2 weeks) requires early diagnosis and immediate chemotherapy if the patient is to survive.

Analysis of the CSF shows decreased glucose and increased protein concentrations. Leukocyte counts may range from several hundred to >20,000 cells/mm^3. Gram stains and bacterial cultures of CSF are negative. A definite diagnosis could be made by demonstration of the amebae in the CSF or in biopsy specimens. Either CSF or sedimented CSF should be placed on a slide under a coverslip and observed for motile trophozoites; smears can also be stained with Wright's or Giemsa stain. CSF, exudate, or tissue fragments can be examined by light microscopy or phase-contrast microscopy. Care must be taken not to mistake leukocytes for actual organisms or vice versa. It is very easy to confuse leukocytes and amebae, particularly when one is examining CSF by using a counting chamber, hence the recommendation to use just a regular slide and coverslip. Motility may vary, so that the main differential characteristic is the spherical nucleus with a large karyosome.

Unfortunately, most cases are diagnosed at autopsy; confirmation of these tissue findings must include culture and/or special staining using monoclonal reagents in IFA procedures. Organisms can also be cultured on nonnutrient agar plated with *Escherichia coli*. In cases of presumptive pyogenic meningitis in which no bacteria are identified in the CSF, the CT appearance of basal arachnoiditis (obliteration of basal cisterns in the precontrast scan with marked enhancement after the administration of intravenous contrast medium) should indicate the possibility of acute PAM.

The amebae can be identified in histology preparations by using indirect immunofluorescence and immunoperoxidase techniques. The organism in tissue sections looks very much like an *Iodamoeba bütschlii* trophozoite, with a very large karyosome and no peripheral nuclear chromatin; the organisms can also be seen with use of routine histologic stains. In general, serologic tests have not been helpful in the diagnosis of this infection. The disease progresses so rapidly that the patient is unable to mount an immune response.

Acanthamoeba spp.

Granulomatous Amebic Encephalitis. Another type of meningoencephalitis, GAE, caused by freshwater amebae may occur as a subacute or chronic disease with focal granulomatous lesions in the brain (Fig. 30.3) (8, 9). Several species of *Acanthamoeba* belonging to several different genotypes cause this insidious and chronic disease,, principally in immunocompromised hosts including persons infected with HIV/AIDS. The route of CNS invasion is thought to be hematogenous, with the primary site being the skin or lungs. In this infection, both trophozoites and cysts can be found in the CNS lesions. A case involving acute onset of fever, headache, and pain in the neck preceded by 2 days of lethargy has been described. Conditions associated with GAE include skin ulcers, liver disease, pneumonitis, diabetes mellitus, renal failure, rhinitis, pharyngitis, systemic lupus erythematosus, and tuberculosis. Predisposing factors include alcoholism, pregnancy, systemic lupus erythematosus, hematologic disorders, AIDS, chemotherapy, radiation therapy, and steroid treatment. This infection has become more widely recognized in AIDS patients, particularly those with a low $CD4^+$ cell count (10). Although *Acanthamoeba* infections stimulate a granulomatous response, the response in AIDS patients is minimal or absent, consistent with the poor immune response in these patients. Examples of specific infections seen in immunocompromised patients are listed in Table 30.6.

The *Acanthamoeba* group also causes keratitis and corneal ulceration. There are a number of published cases, which emphasizes the need for clinicians to consider acanthamoebic infection in the differential diagnosis of eye infections that fail to respond to bacterial, fungal, or viral therapy. These infections are often due to direct eye exposure to contaminated materials or solutions.

Unlike *N. fowleri*, *Acanthamoeba* spp. do not have a flagellate stage in the life cycle, only the trophozoite and cyst (Fig. 30.3). Motile organisms have spinelike pseudopods; however, progressive movement is usually not very evident. There is a wide range in size, with the average diameter of the trophozoites being 30 μm. The nucleus has the typical large karyosome, like that seen in *N. fowleri*. This morphology can be seen on a wet preparation. The cysts are usually round with a single nucleus and also have the large karyosome seen in the trophozoite nucleus. The double wall is usually visible, with the slightly wrinkled outer cyst wall and what has been described as a polyhedral inner cyst wall. This cyst morphology can be seen in organisms cultured on agar plates.

Laboratory examinations similar to those for *N. fowleri* can be used to recover and identify these organisms; the one exception is recovery by culture, which has not proven to be as effective with GAE cases. The most effective approach uses nonnutrient agar plates with Page's saline and an overlay growth of *E. coli* on which the amebae

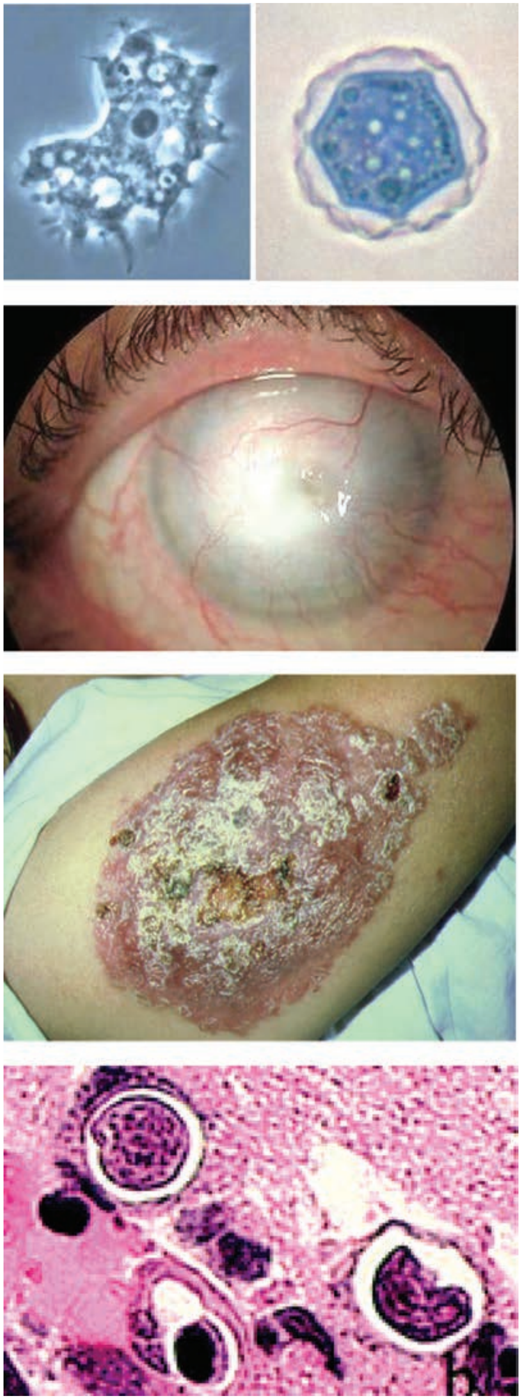


Figure 30.3 (Top, left) *Acanthamoeba* trophozoite (note the sharp, spiky pseudopodia); (right) *Acanthamoeba* cyst (note the hexagonal double wall). (Row 2) *Acanthamoeba* keratitis. (Row 3) Cutaneous *Acanthamoeba* infection. (Bottom) *Acanthamoeba* cysts in brain: case of GAE. Note that cysts are present in tissue, unlike *Naegleria* infections, where only trophozoites are seen in tissue. (Lower three images courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch30.f3

TABLE 30.6 *Toxoplasma gondii* infections in immunocompromised patients[a]

Underlying condition	Disease manifestation and complication	Comments
AIDS and lymphoma	Cerebral toxoplasmosis	Regional CBV (rCBV) determined by perfusion MRI; rCBV reduced throughout toxoplasmosis lesions; all active lymphomas had increased rCBV results; differences were significant; perfusion MRI is a rapid, noninvasive tool that may allow differentiation between cerebral lymphoma and toxoplasmosis
AIDS and lymphoma	Cerebral toxoplasmosis	Knowledge of patient's serostatus does not aid discrimination between lymphoma and toxoplasmosis; single lesions on MRI with focal accumulation of thallium-201 strongly suggest lymphoma; multiple lesions on MRI with uptake ratios of ≥2.9 also suggest lymphoma; uptake ratios of <2.1 do not aid in discrimination
AIDS	CNS disease	Diagnosis of CNS toxoplasmosis often incorrect; another diagnosis is most likely in patients who are anti-*Toxoplasma* seronegative or who are receiving prophylactic TMP-SMX
AIDS	CNS disease and intracranial mass lesions	Neurologic disease is first indication of AIDS in 10–20% of HIV-seropositive individuals; over half will present with intracranial mass lesion; toxoplasmosis is the most common cause in developed countries; data indicate that it is also the most common cause in underdeveloped countries
AIDS	CNS disease and gastrointestinal mastocytosis	Simultaneous occurrence of both conditions and regression after treatment for toxoplasmosis; may suggest a possible relationship between mast cell proliferation and parasitic infection
AIDS	Chorea	Chorea not unusual in AIDS; however, its causes are variable; AIDS-related disease should be considered in young patients presenting with chorea with no family history of movement disorders
AIDS	Risk factors	While risk factors are still somewhat unclear, specific prophylaxis for patients with low CD4 cell counts and high *Toxoplasma* Ab titers is recommended
AIDS	Emerging disease in India (TE)	Management protocol in AIDS patients critical to the diagnosis of TE in patients with CNS symptoms; increasing numbers of AIDS patients require constant consideration of opportunistic infections
AIDS	Immunoblot profiles (TE)	In patients with *Toxoplasma* Ab (ELISA), IgG 27 band or IgG 31 band on Western blot is highly indicative of TE, independent of each other; Western blot positivity provides useful data for improving diagnosis of presumptive TE in HIV-infected patients with suspected TE and positive *Toxoplasma* serology
AIDS	Long-term outcome (TE)	Cerebral infections like TE may negatively influence HIV-1 activity so far latent in the brain
AIDS	Sulfadiazine and obstructive nephropathy	Patients receiving sulfadiazine for cerebral toxoplasmosis can develop obstructive nephropathy; will resolve with alkalic hydration and discontinuation of sulfadiazine
AIDS	High or low doses of TMP-SMX	High doses of TMP-SMX appear to be more effective than low doses for decreased risk for toxoplasmosis in HIV-infected patients; rifampin may reduce the efficacy of TMP-SMX
Immunosuppressive drugs for BMT	Cerebral toxoplasmosis	Clinical diagnosis confirmed by PCR on CSF and blood leukocytes; retrospective testing indicated PCR was positive 52 days prior to onset of clinical symptoms; results highlight value of PCR in early detection of cerebral toxoplasmosis
Immunosuppressive drugs for BMT	TE	In group of 27 BMT patients (allogeneic only) who developed CNS infections, the most common (74%) was TE; one group showed edema but no enhancement, while one group showed the typical MRI pattern with the exception of frequent hemorrhagic transformation
Immunosuppressive drugs for BMT	Cerebral toxoplasmosis reactivation	*T. gondii* infection reactivation occurs predominantly in patients after allogeneic BMT; tends to occur within 3 mo after transplantation; late cerebral toxoplasmosis reactivation may be triggered by a course of corticosteroids (administered for graft-versus-host disease); risk factors should signal reinstitution of prophylactic treatment with TMP-SMX
Immunosuppressive drugs for BMT	MRI problems	In patients with low leukocyte count (poor immunologic response) and/or high doses of immunosuppressive therapy, typical contrast enhancement may be absent (poor visualization of cerebral lesions)
SLE therapy	Cerebral toxoplasmosis	Symptoms of fever, confused state, convulsions consistent with lupus cerebritis; patient died of cerebral toxoplasmosis, which was confused with lupus cerebritis
Non-Hodgkin's lymphoma	TE	Involvement of mediastinum, para-aortic lymph nodes, pleura, peritoneum, bone marrow; patient deteriorated; CSF smear revealed tachyzoites; TE should be considered a potential cause of consciousness disturbance in patients with malignant lymphoma

[a] Adapted from references 26–29. Ab, antibody; BMT, bone marrow transplantation; CBV, cerebral blood volume; MRI, magnetic resonance imaging; SLE, systemic lupus erythematosus; TE, *Toxoplasma* encephalitis; TMP-SMX, trimethoprim-sulfamethoxazole.

feed. There is also evidence to indicate that phosphate-buffered saline can be used. Specimens transported in ameba saline (5.0 ml) and filtered through 13-mm-diameter, 0.22-µm-pore-size cellulose acetate and nitrate filters (Millipore Corp., Bedford, MA) have also been acceptable for organism recovery. The filter is then placed in the center of the nonnutrient agar plate seeded with *E. coli*. Tissue stains are also effective, and cysts can be stained with Gomori's methenamine silver, periodic acid-Schiff (PAS), and calcofluor white.

Although detection of antibodies to *Acanthamoeba* spp. has been confirmed, the usefulness of serologic tests for the diagnosis of these infections has not been proven. Also, these procedures are not generally available. Further work related to the issue of false-positive and false-negative results and interpretive guidelines regarding past exposure or present infection need to be pursued before these diagnostic procedures can be routinely used.

Balamuthia mandrillaris

The leptomyxid ameba *Balamuthia mandrillaris* is relatively uncommon and was originally thought to be another harmless soil organism, unlikely or unable to infect mammals. However, in studies from the CDC, antisera to the leptomyxid amebae have been shown to react in indirect immunofluorescence assays with tissues from both animals and humans with GAE. There have been over 70 cases worldwide, some of which have occurred in AIDS patients (5, 6, 11, 12). The disease is very similar to GAE caused by *Acanthamoeba* spp. and has an unknown incubation period. The clinical course tends to be subacute or chronic and is usually not associated with swimming in freshwater. No characteristic clinical symptoms, laboratory findings, or radiologic indicators have been found to be diagnostic for GAE. The neuroimaging findings show heterogeneous, hyperdense, nonenhancing, space-occupying lesions (Fig. 30.4). Whether single or multiple, they involve mainly the cerebral cortex and subcortical white matter. These findings suggest a CNS neoplasm, tuberculoma, or septic infarcts. Patients complain of headaches, nausea, vomiting, fever, visual disturbances, dysphagias, seizures, and hemiparesis. There may also be a wide range in terms of the clinical course, from a few days to several months. Death can occur from a week to several months after the onset of stroke-like symptoms that can mimic other conditions. Both trophozoites and cysts of *B. mandrillaris* are found in many of the same CNS tissues as are *Acanthamoeba* organisms. Although differentiation of these two organisms in tissue by light microscopy is difficult, in some tissue sections *B. mandrillaris* appears to have more than one nucleolus in the nucleus. Generally, electron microscopy

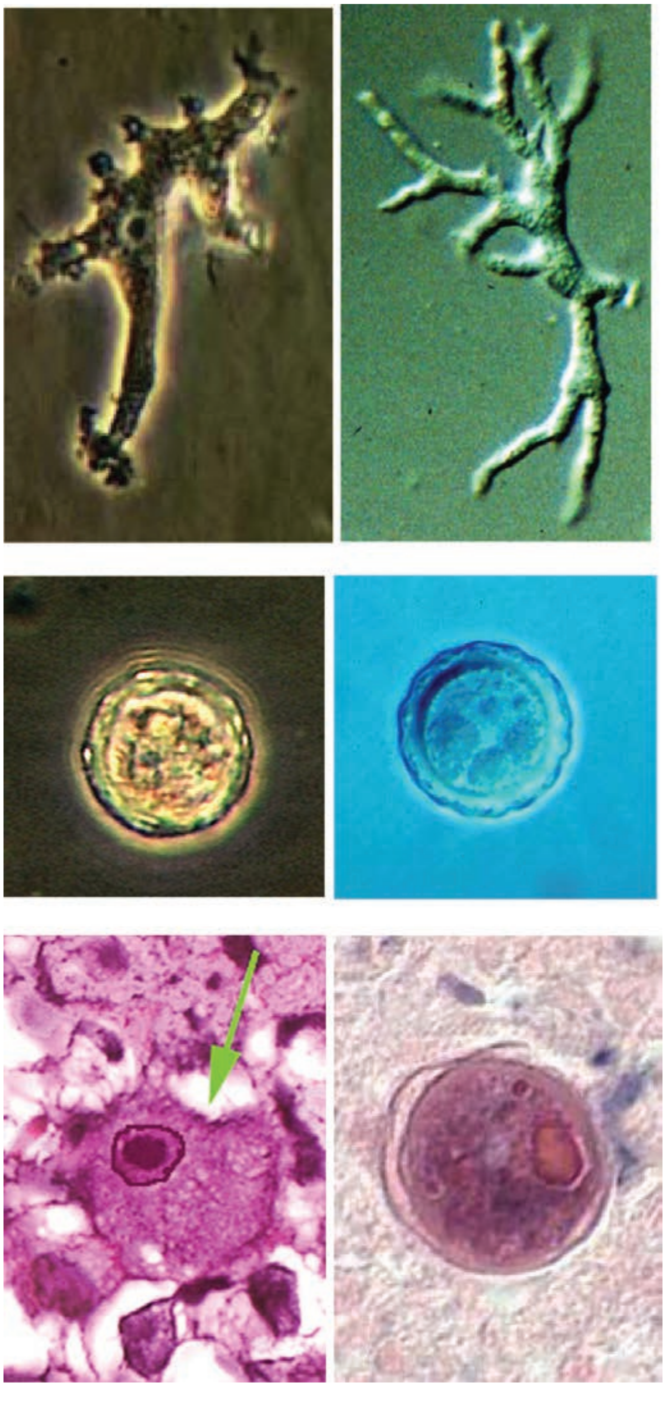


Figure 30.4 *Balamuthia mandrillaris*. (Top) Trophozoites. (Middle) Cysts. (Bottom, left) Trophozoite in brain tissue; (right) cyst in brain tissue (courtesy of the CDC Public Health Image Library; lower right from the University of Kentucky Hospital, Lexington, KY). doi:10.1128/9781555819002.ch30.f4

and histochemical methods are required for definitive identification of *B. mandrillaris*.

Attempts to isolate the organisms from some humans with GAE have not been successful, and leptomyxid amebae do not grow well on *E. coli*-seeded nonnutrient agar plates. In the diagnostic laboratory, these organisms can be cultured using mammalian cell cultures; some success has been obtained with monkey kidney cells and with MRC, HEp-2, and diploid macrophage cell lines. The trophozoites have extensive branching and a single nucleus (occasionally binucleate forms are seen) with a central karyosome, and they measure 15 to 60 µm. Occasionally, a few elongated forms with several contractile vacuoles are seen. The cysts have a single nucleus (occasionally binucleate forms are seen), have the typical double wall with the outer wall being thick and irregular, and measure 15 to 30 µm. Using primary cultures of human brain microvascular endothelial cells, amebae have been isolated from the brain and CSF (13). Serum antibodies to *B. mandrillaris* have been found in both adults and children; however, testing is generally limited to the CDC, where PCR has also been used for the detection of mitochondrial 16S rRNA gene DNA from the amebae in clinical specimens (14).

From the information presented above, it is clear that *B. mandrillaris*, formerly regarded as having no pathogenic potential, will continue to be identified as the etiologic agent of fatal meningoencephalitis in humans as well as animals. This opportunistic infection may also continue to cause disease in individuals with AIDS.

Sappinia sp.

Sappinia pedata and *Sappinia diploidea* are newly discovered human pathogens of the CNS which can cause amoebic encephalitis in humans. *Sappinia* has a worldwide distribution and has been isolated from elk and buffalo feces, soil contaminated with bovine feces, decaying ground plant litter, and freshwater. The life cycle of *Sappinia* consists of two stages, a trophozoite and a cyst (Fig. 30.5). The first case of amoebic encephalitis caused by *Sappinia* sp. occurred in a previously healthy immune-competent adult male who survived the infection. The route of infection is thought to be by inhalation through the nasopharynx or by hematogenous spread to the brain.

In the first case reported, a sinus infection occurred prior to the onset of symptoms. The patient developed nausea, vomiting, bifrontal headache, photophobia, and blurry vision. A loss of consciousness occurred for a brief period (6). Brain images revealed a solitary mass in the posterior left temporal lobe that was made up of necrotizing hemorrhagic

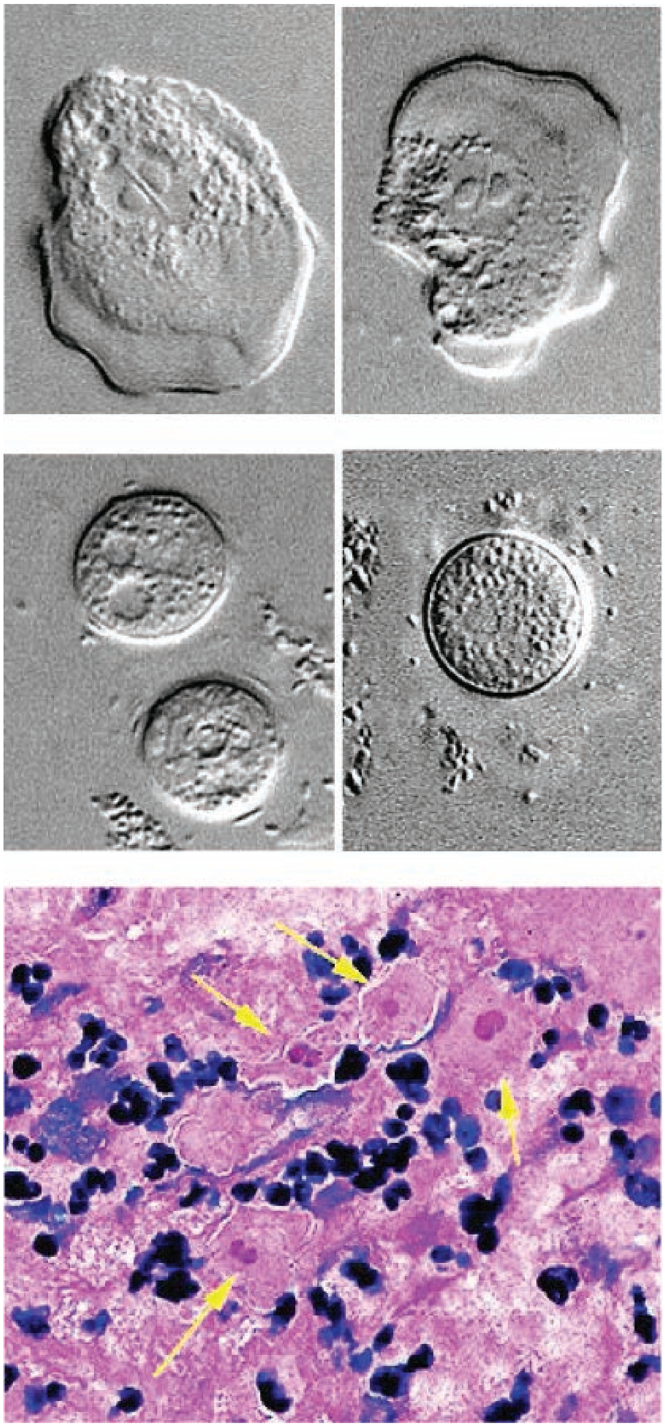


Figure 30.5 *Sappinia* sp. (Top) Trophozoites viewed under differential interference contrast (DIC); note there are two nuclei. (Middle) Cysts viewed under DIC. (Bottom) Four trophozoites (yellow arrows) of *S. pedata* in brain tissue, H&E stain; in three of the amebae, two nuclei are visible (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch30.f5

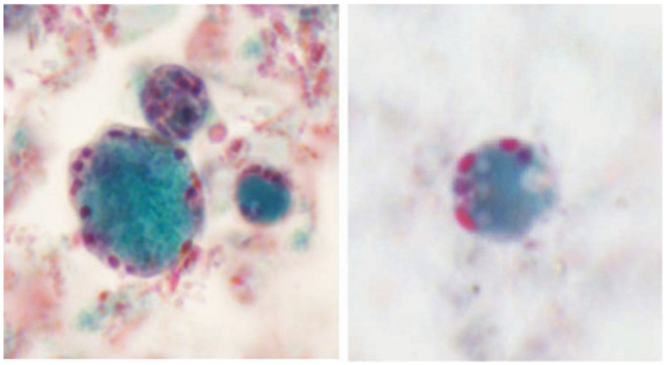
inflammation containing free-living amebae. Immunofluorescence microscopy showed that the organism was not one of the known species of ameba causing encephalitis. Trophozoites had a double nucleus, and transmission electron microscopy confirmed that they contained two nuclei closely apposed along a flattened surface. The nuclear structure and other unique structural features led to the diagnosis of *S. diploidea* encephalitis (15). The patient survived after surgical excision of the tumor-like mass in the brain and treatment using azithromycin, intravenous pentamidine, itraconazole, and flucytosine (15). However, the amoeba that caused this encephalitis was originally identified as *S. diploidea* but has now been identified as *S. pedata* by molecular techniques. The assay specific for *Sappinia* can be incorporated into the multiplex PCR assay that distinguishes *Acanthamoeba*, *B. mandrillaris*, and *N. fowleri* for simultaneous detection of the four genera of free-living amebae that cause infections in humans (16). This assay was able to detect one single ameba per sample, as determined with CSF spiked with diluted cultured amebas. *Sappinia* can be cultured on nonnutrient agar plates with a bacterial overlay, as well as tissue culture cells.

Blastocystis spp.

Intestinal protozoa are becoming increasingly recognized as significant pathogens in immunocompromised hosts. Microsporidia and *Blastocystis* have also emerged as important players, due in part to improved molecular diagnostic assays. *Blastocystis* spp. are among the most common intestinal protozoan parasites found in humans (Fig. 30.6). The prevalence of *Blastocystis* ranges from approximately 10% in developed countries to 60% in developing countries.

Blastocystis has been traditionally named *Blastocystis hominis* when isolated from human fecal materials. However, recent phylogenetic analyses suggest limiting its name to "*Blastocystis* species" because of their genetic diversity. This parasite has been considered as a species complex comprising 13 subtypes, of which at least 9 have been found in humans (17). Furthermore, they exhibit wide genetic diversity that may result in the assignment of different species to the subtypes. The confirmation of their species status and determination of virulence and pathogenic profiles might explain why some patients remain asymptomatic while others have clinical symptoms.

Figure 30.6 *Blastocystis* sp. Central body forms, Wheatley's trichrome stain. doi:10.1128/9781555819002.ch30.f6



Disagreements regarding its pathogenicity led to remarkable findings on both phenotypic and genotypic characteristics of asymptomatic and symptomatic human-derived *B. hominis* isolates (18). Certain subtypes of this organism are associated with intestinal disorders including irritable bowel syndrome. Cases of invasive intestinal infections and skin rashes have been attributed to infection by *Blastocystis* sp. in immunocompetent individuals, suggesting that it is an emerging parasite with pathogenic potential. In a recent case, a 22-year-old female patient complained of pain in the left hypochondrium. Ultrasonography and abdominal CT scans revealed two splenic cysts. The cyst fluid analysis demonstrated numerous *Blastocystis* sp.; PCR and DNA sequencing analyses confirmed the presence of *Blastocystis* subtype 3 (19).

The genome of *Blastocystis* subtype 7 encodes proteases, hexose digestion enzymes, lectins, protease inhibitors, and glycosyltransferases besides several proteins that are predicted to be secreted. The roles of some of these proteins are known in other parasites with direct connections to their pathogenicity in processes such as host cell invasion, excystation, metabolism, cytoadherence, and other virulence functions (20). Cysteine proteases from *Blastocystis* can increase epithelial permeability, and cysteine proteases are important enzymes for host invasion and infection and are well recognized as virulence factors in pathogenic protozoa (20).

Giardia lamblia

Giardia lamblia (*duodenalis*, *intestinalis*) is a flagellate commonly found in many parts of the world (Tables 30.3 and 30.4; Fig. 30.7). Although a number of papers on the nomenclature of *Giardia duodenalis* replacing *G. lamblia* or *G. intestinalis* have been published, apparently this change has not been formally recognized by the International Code of Zoological Nomenclature. Therefore, we will maintain the name *G. lamblia* for this edition. An infection with this organism can be transmitted via the cyst form in both water and food contaminated with fecal material, as can *E. histolytica* infections. Various conditions that have been associated with giardiasis in the compromised patient include hypogammaglobulinemia, protein or caloric malnutrition, previous gastrectomy, histocompatibility antigen HLA YB-12, gastric achlorhydria, blood group A, differences in mucolytic proteins in immunoglobulin deficiencies, and reduced secretory IgA levels in the gut (Table 30.2). Generally, adults and children are mentioned in relation to giardiasis; however, it is important to remember that the elderly, in both tropical and temperate zones, are also susceptible. These patients tend to be somewhat immunodepressed due to age and may be overlooked as having giardiasis.

Although patients with HIV infection have also been found to have giardiasis, the infection does not appear to be more severe among this group, regardless of the $CD4^+$ cell count (21). In some groups, the prevalence of *G. lamblia* is greater for HIV-infected subjects (22, 23). Both humoral and cellular immune responses play a role in acquired immunity, and the mechanisms involved remain somewhat unclear. However, the immune response to intestinal parasites, including *G. lamblia*, might be a risk factor for HIV/AIDS and tuberculosis (24). It is well known that diagnosing intestinal parasites in HIV/AIDS patients is necessary, especially in those who are chronic alcoholics or are not receiving antiretroviral treatment. With progressive immunosuppression following reduced $CD4^+$ counts, the risk of symptomatic *Giardia* infections is increased. However, giardiasis is not considered a major cause of enteritis in HIV-infected patients.

Multiple infections tend to be more common among renal transplant recipients. This highlights the importance of testing for intestinal parasites among this group. Routine examinations of stool samples for parasites would significantly benefit renal transplant recipients by helping to reduce severe infections. Symptomatic giardiasis has been observed in X-linked infantile congenital hypogammaglobulinemia (Bruton's syndrome) and also in the common variable (late-onset) acquired hypogammaglobulinemia. Immunocompromised patients such as nephrotic syndrome children receiving corticosteroids, protein-calorie malnutrition patients, patients with cases of marasmic kwashiorkor, and lymphoma patients have been found to be significantly more at risk for *Giardia* infection (25).

Symptoms of giardiasis may range from none to the malabsorption syndrome, which is characterized by gas,

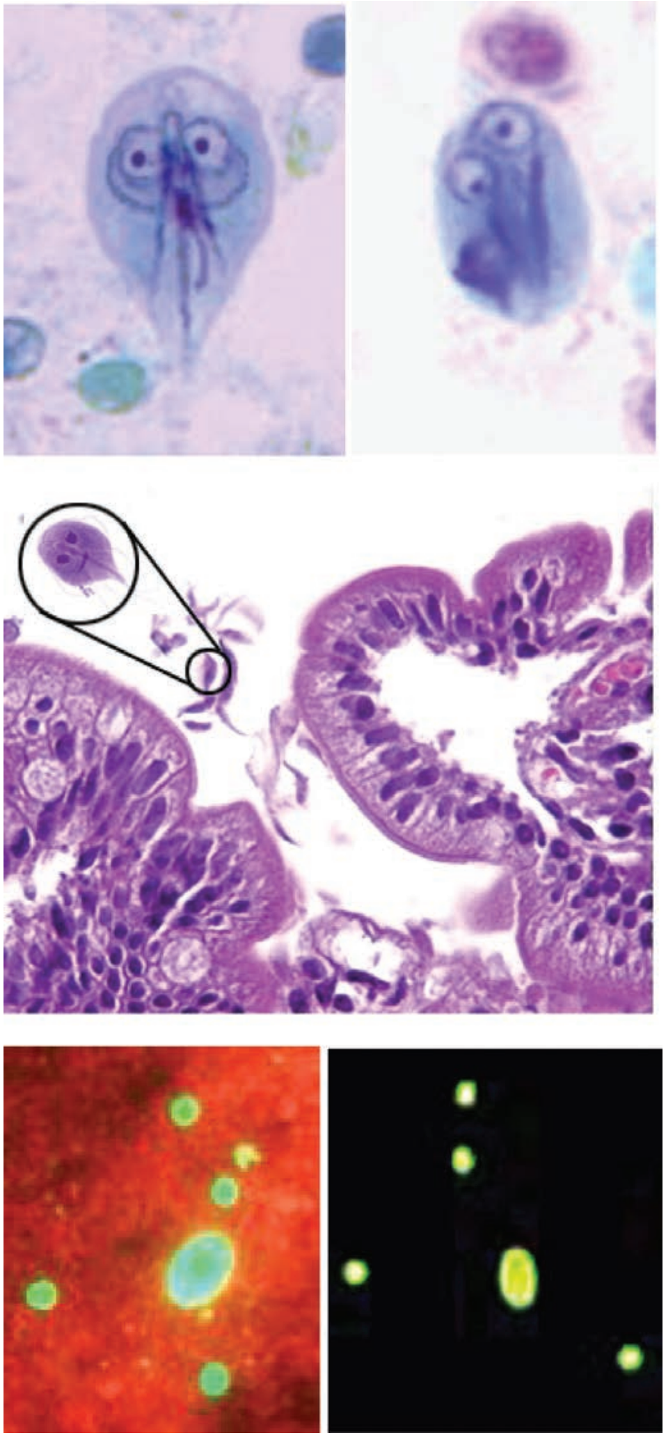


Figure 30.7 (Top, left) *Giardia lamblia* trophozoite (note two nuclei, curved median bodies, and linear axonemes); (right) *G. lamblia* cyst. (Middle) Multiple *Giardia* trophozoites seen in gastrointestinal tract; note the enlargement (courtesy of Medical Sciences, Indiana University, http://medsci.indiana.edu/c602web/602/c602web/nutrit/docs/cas4_giard.htm). (Bottom) *G. lamblia* cyst (large) and *Cryptosporidium* spp. oocysts (small) demonstrating fluorescence in the fecal FA immunoassay. Note in the left image that the background demonstrates use of the counterstain, while in the right image no counterstain was used.
doi:10.1128/9781555819002.ch30.f7

bloating, frothy green foul-smelling stools, and abdominal pain. The organism is reasonably easy to identify. The trophozoite form is teardrop shaped, and the flagella and sucking disk are easily visible. On side view, the trophozoite often looks somewhat like the curved end of a spoon. Trophozoites tend to stain pale, while the cysts usually stain darker and are easier to identify. Cysts are round to oval, with long axonemes and curved median bodies being visible. Normally, cysts can be seen and identified on wet mount preparations from concentrated material and confirmed by using the permanent stained smear.

In contrast to *E. histolytica*, *G. lamblia* inhabits the duodenal area of the intestine, tends to adhere very tightly to the mucosa, and can be very difficult to recover, even after a series of five or six stool examinations. For this reason, other techniques, such as the Entero-Test string capsule, duodenal aspirate, or biopsy, may have to be used. Fecal immunoassays are also recommended for the diagnosis of giardiasis; they are more sensitive than the routine ova and parasite examination.

Toxoplasma gondii

Toxoplasma gondii is worldwide in distribution, and serologic data indicate that human infections are common, although most infections are benign or cause no symptoms (Tables 30.3 and 30.4). Serologic evidence from throughout the United States indicates that anywhere from 20 to 70% of the population are serologically positive for *T. gondii*. The percentage of serologically positive patients tends to be higher in the tropical areas of the world.

Toxoplasmosis can vary from an asymptomatic, self-limiting infection to a fatal disease, as seen in patients with congenital infections or in debilitated patients in whom underlying conditions may influence the final outcome of the infection (Table 30.7). In immunocompromised patients, the infection most often involves the nervous system, with diffuse encephalopathy, meningoencephalitis, or cerebral mass lesions (Fig. 30.8). In one study, multiplex nested PCR analysis was used to determine the genotypes of parasites in CSF; 8 of 10 human HIV-positive patients had infections with type I strains, even though this lineage is normally uncommon in humans and animals (26). Although gastric toxoplasmosis is rare, in some patients with AIDS it is the primary manifestation (27).

Underlying conditions associated with toxoplasmosis in the compromised host include various types of malignancies, such as Hodgkin's disease; non-Hodgkin's lymphomas, leukemias, or solid tumors; collagen vascular disease; and organ transplantation. We must also add to this category patients with AIDS. Over 50% of such patients with toxoplasmosis have alterations of mental status, motor impairments, seizures, abnormal reflexes, and other neurologic symptoms. Studies have shown that even among compromised patients, 80% of those receiving chemotherapy for toxoplasmosis experienced significant clinical improvement or complete remission; however, in those with AIDS, therapy must be continued for long periods to maintain a clinical response.

Toxoplasma encephalitis has been reported as a life-threatening opportunistic infection among patients with AIDS. The overall prognosis can be poor, with the median survival following initiation of therapy being 4 months. A clinical relapse of *Toxoplasma* encephalitis has been reported to occur in 50% of patients discharged from hospital. In AIDS patients who may not receive prophylaxis for *Pneumocystis jiroveci* pneumonia, almost half of the *Toxoplasma*-seropositive patients can develop CNS toxoplasmosis. This figure is considerably higher than previously thought for the development of CNS involvement. Statistics indicate that, overall, approximately 2.5% of AIDS patients have been diagnosed with cerebral toxoplasmosis. Information on toxoplasmosis and the immunocompromised host can be seen in Table 30.7.

In seropositive allogeneic hematopoietic stem cell transplant recipients, the hazard of *T. gondii* reactivation is generally high. The donor's seronegative status seems to contribute to this risk, as approximately 80% of all reported events of toxoplasmosis are observed in cases of donor/recipient serological mismatch. Clinical symptoms of reactivation are usually observed within 2 to 4 months posttransplant. Additional factors that may contribute to early and heavy *T. gondii* dissemination and rapid clinical deterioration include the lack of posttransplantation prophylaxis in an immunosuppressed seropositive recipient. Other contributing factors may involve receipt of a graft from an atypical genotype of *T. gondii* (28).

Humans usually become infected with *T. gondii* by ingesting raw or poorly cooked meats. Another means of transmission is ingestion of infected oocysts found in cat feces. It has been recommended that litter boxes be cleaned daily, with feces disposed of in the toilet and the pans disinfected with boiling water. Since we know that transplacental transmission can occur during an acute but perhaps undiagnosed maternal infection, pregnant women, unless they have serologic evidence of previous infection, should avoid contact with cats whose source of food is not controlled and should not empty litter boxes. Uterine or neonatal infections can give rise to a number of serious sequelae, including microcephaly and hydrocephalus.

In humans, *T. gondii* is found in two different stages. The actively proliferating intracellular forms are called trophozoites or tachyzoites and are crescent shaped. Many different tissues may be parasitized by these organisms,

TABLE 30.7 *Cryptosporidium* infections in immunocompromised patients[a]

Underlying condition	Disease manifestation	Comments
AIDS	Diarrhea and possible dissemination	Parasites associated with 14% of acute diarrheal episodes (<28 days) and 35% of chronic episodes (>28 days); parasite associated with diarrhea in 14% of patients when CD4$^+$ counts are ≥200 cells/µl and in 27% when counts are <200 cells/µl; *Cryptosporidium* associated with 5% of acute episodes and 13% of chronic episodes of diarrhea; *Cryptosporidium* likely to be associated with diarrhea as immunosuppression worsens and with chronic rather than acute diarrhea
AIDS	Diarrhea	Attack rate 14% among HIV-negative individuals and 31% among HIV-positive individuals (varies due to CD4 cell count); chronic symptoms in 15% (<150 CD4 cells/µl at onset); 14% had antibody before outbreak, 51% developed specific antibodies during outbreak; clinical manifestations of cryptosporidiosis strongly influenced by level of HIV-induced immunosuppression
AIDS	Pancreatitis	Acute or chronic pancreatitis associated with *Cryptosporidium* infection; abdominal pain resistant to analgesics, increased amylase level in serum; abnormalities on both sonography and CT; papillary stenosis in all patients; oocysts found in both bile and stool; viral cultures negative; several antibiotic protocols ineffective
AIDS	Gastric involvement	Of 24 individuals, 34% positive for *Cryptosporidium*, 16 (89%) of these had parasites in gastric epithelium; most patients did not show specific symptoms indicating presence in stomach; gastric involvement probably more frequent than expected; no clear correlation between gastric location and related clinical or pathologic features
AIDS	Diarrhea	22 *Cryptosporidium* isolates (Kenya, Switzerland, United States); 4 distinct genotypes identified (*C. parvum*, human genotype; *C. parvum*, cattle genotype; *C. felis*; and *C. meleagridis*, turkey); immunocompromised individuals susceptible to wide range of *Cryptosporidium* spp. and genotypes; full public health ramifications not known
AIDS	Diarrhea	*C. parvum* genotype subgroupings correlate to some extent with infectivity and suggest that additional heterogeneity is present within the subgroups
AIDS	Diarrhea	Treatment of HIV-1-associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy; combination therapy that includes protease inhibitors can restore immunity to both organisms and result in clinical, microbiological, and histologic responses; persistent CD8 cell and macrophage infiltrate and short time to relapse with declining CD4 lymphocyte counts suggest that neither infection was eradicated
AIDS	Diarrhea due to contaminated tap water	Risk assessment model applied to New York City population; calculated number of tap water-related cases per year was 6 (non-AIDS subgroups) or 34 (AIDS subgroups)
AIDS, pediatrics	Diarrhea	Some 81% of HIV-infected children with persistent diarrhea excreted *Cryptosporidium*, microsporidia, or both organisms, compared with only 10% of children with persistent diarrhea testing negative for HIV; 74% of isolates were *C. hominis*, 17% were *C. parvum*, and 8% were a mixture of the 2 or others
All causes	Diarrhea due to zoonotic transmission	Presence of *Cryptosporidium* in cats (5%) (in addition to other organisms such as *Giardia* spp., *Toxocara cati*, *Salmonella*, *Campylobacter*); enteric zoonotic organisms detected in total of 13% of cats studied; cats in homes of immunocompromised humans should be evaluated for zoonotic organisms
Hodgkin's lymphoma	Diarrhea	Immunocompromised prevalence of 14.3%; control group had 5.6%
Immunosuppressive therapy for pediatric oncology	Diarrhea	In contrast to studies of adult oncology patients, pediatric oncology patients in some areas appear to be at low risk for cryptosporidiosis
Immunosuppressive drugs for kidney transplantation, chronic renal insufficiency	Diarrhea	*Cryptosporidium* found in 35% (renal transplant group), 25% (patients on hemodialysis), and 17% (control group with systemic arterial hypertension) of patients; when number of positive fecal samples is taken into account, there is a significantly higher frequency in renal transplant patients
Immunosuppressive drugs for kidney transplantation	Diarrhea	*Cryptosporidium* present in 19% of patients, *Blastocystis hominis* in 39%; symptomatic infections with *Cryptosporidium* significantly higher in transplant patients than controls
Immunosuppressive drugs for liver transplantation	Diarrhea	Primary hypogammaglobulinemia, chronic hepatitis C, hepatic failure; 14 mo posttransplantation, *Cryptosporidium* diarrhea; no response to therapy; patient died
Immunosuppressive drugs for solid-organ transplantation[b]	Diarrhea	Included 3 liver transplants and 1 small bowel transplant; all 4 patients resolved cryptosporidiosis; while cryptosporidiosis is a nonlethal complication, it allows physician to monitor degree of immunosuppression and relationship to infection

[a] Adapted from references 25 and 31. CT, computed tomography.
[b] Excludes kidney transplantation.

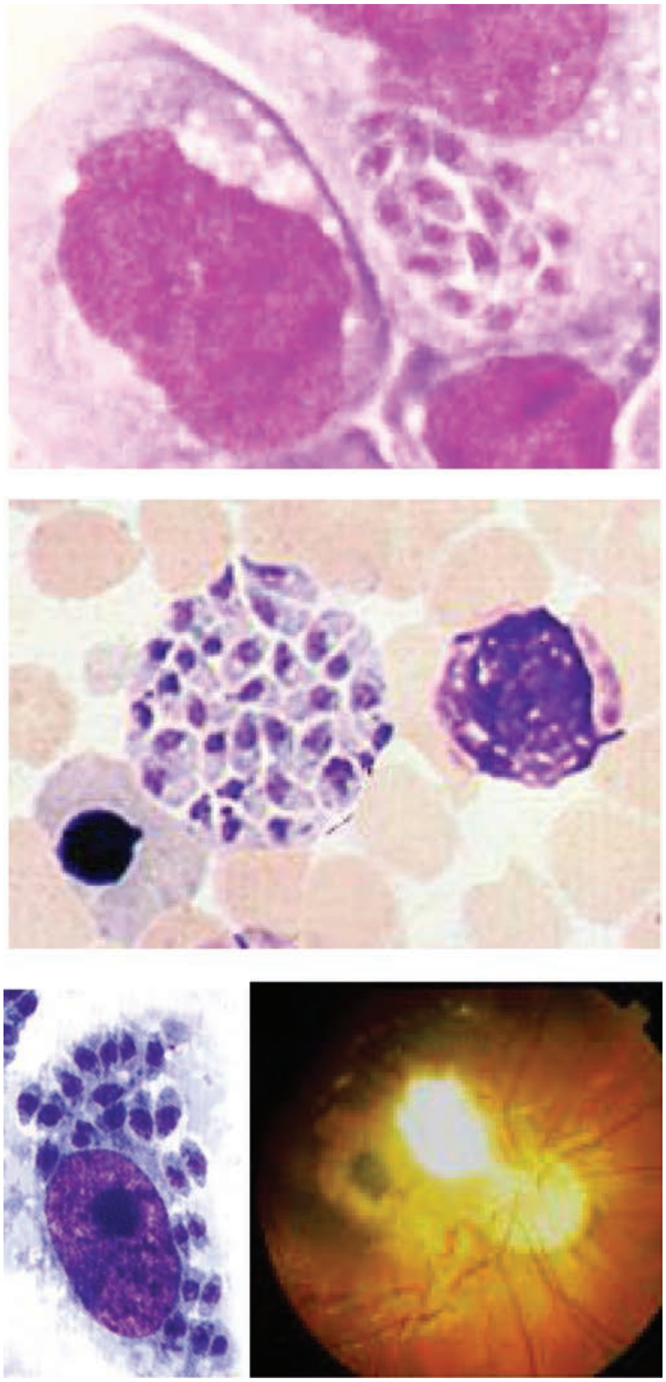


Figure 30.8 *Toxoplasma gondii*. (Top) Tissue cyst containing 8 to 20 parasites. (Middle) Organisms in bone marrow. (Bottom, left) Tachyzoites in tissue culture; (right) granuloma in eye. doi:10.1128/9781555819002.ch30.f8

particularly lung, heart, lymphoid organ, and CNS tissues. The resting forms or cyst stages are found in the tissues and contain the more slowly developing bradyzoites.

Toxoplasmosis can be diagnosed by using various serologic procedures, histologic findings from the examination of biopsy specimens, and isolation of the organism, either in a tissue culture system or by animal inoculation. Individuals with positive serologic tests have been exposed to *Toxoplasma* organisms and may have the resting stages within their tissues. This is why histologic identification of the organism and its recovery either in a tissue culture system or by animal inoculation may be misleading, since the organisms could be isolated yet might not be the etiologic agent of disease. Thus, serologic tests have been recommended as the diagnostic tests of choice. One of the first serologic tests was the Sabin-Feldman dye test, a reaction based on the fact that serum antibodies to *T. gondii* will affect the organisms so that they become refractory to staining with methylene blue. However, since this test requires the use of living *Toxoplasma* trophozoites, most laboratories do not provide it for clinical use.

In an immunodeficient or immunosuppressed patient, a presumptive diagnosis of toxoplasmosis can be made by observing an elevated serologic titer and the presence of the clinical syndrome, which would include neurologic symptoms. However, in certain patients with monoclonal gammopathies, titers to *T. gondii* may be extremely high but may not reflect the cause of the clinical condition. One of the most common diagnostic tests used for toxoplasmosis is the IFA procedure. Definitive diagnosis is usually made when there is a rising titer, an IgM-IFA titer of at least 1:64, or an IgM-ELISA titer of 1:256. A finding that the Sabin-Feldman dye reaction is higher in CSF than in serum is also significant but not that common. An elevated serologic titer in conjunction with demonstration of the actual trophozoites or isolation of the organisms from cerebrospinal fluid is very significant.

A new possibility in *T. gondii* serodiagnosis is the selection and use of molecular markers, which are proteins characteristic of the parasite's tachyzoite or bradyzoite stage that are able to recognize the specific antibodies from acute or chronic human sera. Promising results show that the use of a specific recombinant protein identifies the phase of the disease during the testing of a single serum sample (29).

Cryptosporidium spp.

Although it has been recognized as an animal parasite for many years, one of the newer parasitic organisms infecting humans, particularly compromised patients, is *Cryptosporidium*, a cause of diarrhea (Tables 30.3 and 30.4; Fig. 30.9) (25, 30, 31). *Cryptosporidium* differs from other coccidia that infect warm-blooded vertebrates in that the developmental stages do not occur deep within host cells but are confined to an intracellular, extracytoplasmic loca-

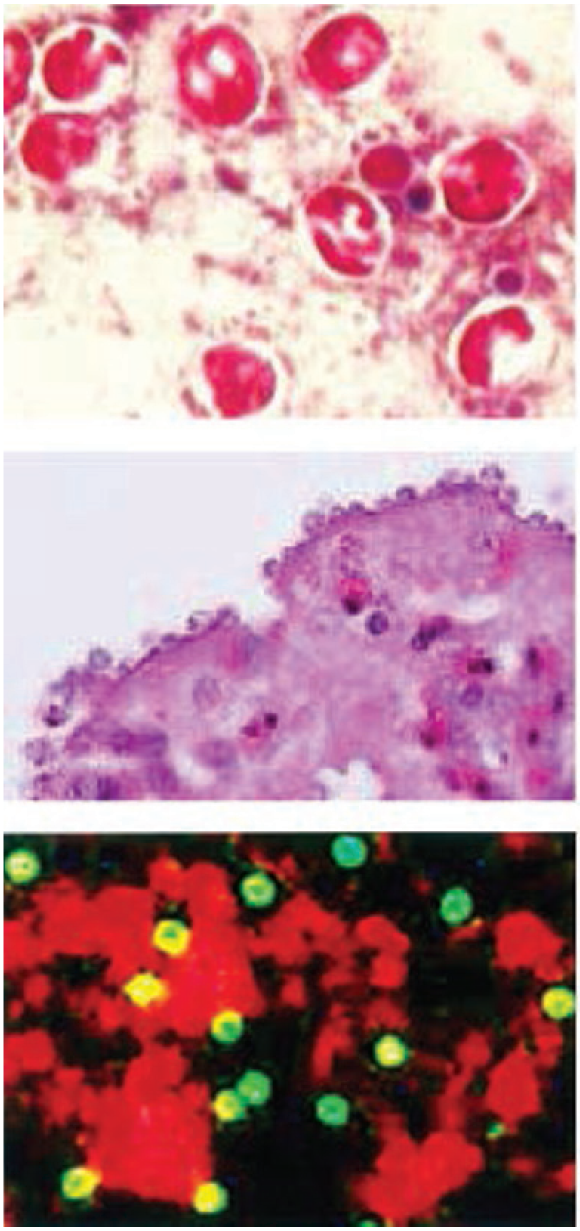


Figure 30.9 *Cryptosporidium.* (Top) Oocysts stained using the modified acid-fast stain; note the spherical shape. Oocysts measure 4 to 6 µm and some contain sporozoites that are visible. (Middle) Organisms visible along the brush border of intestinal mucosa. (Bottom) FA procedure showing fluorescent apple-green oocysts; counterstain present.
doi:10.1128/9781555819002.ch30.f9

tion. Each stage is within a parasitophorous vacuole of host cell origin. What was previously called *C. parvum* and was thought to be the primary *Cryptosporidium* species infecting humans is now classified as two separate species, *C. parvum* (mammals, including humans) and *C. hominis* (primarily humans). Differentiation of these two species based on oocyst morphology is not possible.

Studies have shown that calves and perhaps other animals may serve as potential sources of human infections. Kittens, puppies, and rodents are possible reservoir hosts, since they are easily infected with human *Cryptosporidium*. Person-to-person transmission is also likely and may occur through direct or indirect contact with stool material in the environment and the hospital setting.

This organism is worldwide in distribution, and although the first cases in the literature indicated that the patients were immunosuppressed or immunodeficient, current literature reports numerous infections and outbreaks in patients with a normal immune system capability. In patients with a normal immune system capability, cryptosporidiosis is self-limiting. However, in certain compromised patients, the diarrhea is very severe and prolonged, with death being the eventual outcome (Tables 30.4 and 30.7). Clinical symptoms include nausea, low-grade fever, abdominal cramps, anorexia, and 5 to 10 watery stools per day. Fluid loss in these patients is significant and has been reported to be as great as 17 liters/day.

Although many therapeutic regimens have been tried, there is no completely satisfactory therapy for cryptosporidiosis in humans. Paromomycin, clarithromycin, nitazoxanide, and hyperimmune bovine colostrum are among the drugs being tried in different regimens; the current recommendation is nitazoxanide (30). However, no drug has proven efficacy in cryptosporidiosis in immunosuppressed patients. For HIV-infected patients, potent antiretroviral therapy is the mainstay of treatment. Nitazoxanide (treatment duration of 5 to 21 days), paromomycin, or a combination of paromomycin and azithromycin may be tried to decrease diarrhea and recalcitrant malabsorption of antimicrobial drugs, which can occur with chronic cryptosporidiosis (30). Specific recommendations for immunocompromised patients in avoiding potential risk situations for acquiring infection with *Cryptosporidium* spp. can be found in Table 30.8.

Since the organisms live in the microvillous surface of the mucosal epithelial cells of the intestine, diagnostic techniques have included histologic examination of intestinal biopsy material, scanning and transmission electron microscopic examination of duodenal aspirates and biopsy specimens, and the examination of stool specimens for oocysts.

Although biopsy techniques have been used to recover and identify the organisms in tissue, one problem with a tissue specimen is that the specific area where the organisms reside may not be subjected to biopsy. The examination of stool material for oocysts by using an acid-fast technique or immunoassays allows one to screen a sample from the entire intestinal tract. It is considerably cheaper to examine

TABLE 30.8 Prevention of cryptosporidiosis in immunocompromised patients[a]

Preventive measure	Comments
Hand washing	Prior to eating and food preparation; after touching children in diapers; after touching clothing, bedding, toilets, or bed pans from anyone with diarrhea; after gardening; after touching pets or other animals; after touching or coming in contact with anything contaminated with human or animal stools (includes dirt); after removing gloves to perform any of the above activities
Safe sex	Always wash hands after touching partner's anal or rectal area
Avoidance of farm animals	Particularly important for contact with young animals (especially lambs and calves); wash hands thoroughly; avoid touching stools of any animal; after potential exposure, clean shoes (avoid having someone who is HIV infected perform this task)
Avoidance of pet stools	If HIV infected, avoid cleaning litter boxes or cages and disposing of stools; very important to avoid if animals are less than 6 months old; have puppies or kittens (if less than 6 months old) tested for *Cryptosporidium* before bringing them home; adult cats have also been found to be positive; have any pet tested that develops diarrhea
Precautions while swimming	Avoid swallowing water when in lakes, rivers, pools, or hot tubs; organisms not killed by routine chlorination; oocysts can also survive several days in salt water, so avoid swimming in polluted ocean water
Washing and cooking food	Wash well all fruits and vegetables if eating uncooked; use safe water for washing food; peel fruit; avoid unpasteurized milk or dairy products; cooking kills *Cryptosporidium*; cooked or packaged food is safe unless handled by someone infected with the organism
Water for drinking	Do not drink directly from lakes, rivers, streams, or springs; may want to avoid unboiled tap water (includes water and/or refrigerator ice-maker ice); boil water (rolling boil for 1 min sufficient to kill organisms), filter with appropriate filters (look for "reverse osmosis," "absolute 1 micron," "Standard 53," and the words "cyst reduction" or "cyst removal"); home distiller can be used; store water as for boiled water
Bottled water	Bottled water labels reading "well water," "artesian well water," "spring water," or "mineral water" do not guarantee that the water does not contain *Cryptosporidium*; however, water that comes from protected well or spring water sources is less likely to be contaminated than bottled water from rivers and lakes
Drinks	Canned or bottled soda, seltzer, fruit drinks, and steaming hot tea and coffee are safe; fountain drinks, fruit drinks mixed with tap water from concentrate, and iced tea or coffee may not be safe

[a] Adapted from information prepared by the interagency Working Group on Waterborne Cryptosporidiosis.

a stool specimen than to perform endoscopy or histologic testing. *Cryptosporidium* oocysts in the stool range from 4 to 6 µm in diameter and can be difficult to identify. One must also remember that even in patients with typical watery diarrhea, the numbers of organisms passed can be quite variable.

Respiratory cryptosporidiosis has also been detected in AIDS patients. Sputum specimens should be submitted in 10% formalin or as a fresh specimen and can be examined by the same techniques as used for stool samples.

Some techniques have included sugar flotation, formalin sedimentation, Giemsa stain, trichrome, PAS, methenamine silver, acridine orange, auramine-rhodamine, iodine, modified acid-fast, Kinyoun and Ziehl-Neelsen acid-fast, and immunoassay techniques. The most widely used techniques are modified acid-fast stains, Kinyoun's methods, and fecal immunoassays. Specimens preserved in polyvinyl alcohol do not stain well in the acid-fast techniques.

Because other organisms may also fluoresce when auramine or the auramine-rhodamine combination is used, presumptive positive specimens should be confirmed by using one of the acid-fast methods. *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* fluoresce with auramine-rhodamine and are acid-fast positive. *Cryptosporidium* oocysts are fully mature and infective at the time they are passed in the stool. One can occasionally see the four sporozoites with the dark-staining residual body in the fully developed oocyst.

Immunoassay procedures for the direct detection of *Cryptosporidium* antigen or oocysts in fecal specimens have proven to be much more sensitive than the routine acid-fast stains. Since acid-fast stains do not always consistently stain all oocysts, the increased sensitivity and specificity of these immunoassays provide excellent testing methods. Enzyme immunoassay, solid-phase immunochromatographic assay in cartridge format, and fluorescent antibody methods are currently available. The choice of method depends on a number of variables and differs among diagnostic laboratories. All of the fecal immunoassays are comparable in terms of specificity and sensitivity. Molecular tests are becoming more widely available, including some gastrointestinal panels.

Since *Cryptosporidium* has been recovered from patients with AIDS, it has been recommended that stool specimens be submitted in 5 or 10% formalin, sodium acetate-acetic acid-formalin, or the newer single-vial collection systems for processing. However, each laboratory must confirm that the fixatives selected are compatible with diagnostic procedures performed, including the ova and parasite examination and immunoassays; not all single-vial systems are compatible with diagnostic immunoassay reagents. Laboratories should use standard precautions when working with these stool specimens. When specimens are centrifuged, capped centrifuge tubes should be used to prevent the formation of aerosols. Also, if possible, specimen processing should be performed in a biological safety cabinet.

Cyclospora cayetanensis

Cyclospora cayetanensis organisms are acid-fast-variable coccidia and have been found in the feces of immunocompetent travelers to developing countries, immunocompetent subjects with no travel history, and patients with AIDS (Fig. 30.10) (32–36). The life cycle has been confirmed, but information on potential reservoir hosts has yet to be defined; however, it appears that in some areas the human is the only host (Table 30.9). Outbreaks linked to contaminated water and various types of fresh produce (raspberries, basil, baby lettuce leaves, and snow peas) have been reported.

Oocyst maturation takes approximately 7 to 15 days, so the mature stage may not have been seen in human specimens. The oocyst contains two sporocysts, each containing two sporozoites, a pattern which places these organisms in the coccidian genus *Cyclospora*. Unsporulated oocysts are passed in the stool, and sporulation occurs within approximately 1 to 2 weeks. In patients who have *Cyclospora* oocysts in their stool specimens, two types of meronts and sexual stages have been found within the jejunal enterocytes.

There is generally 1 day of malaise and low-grade fever, with rapid onset of diarrhea of up to seven stools per day. There may also be fatigue, anorexia, vomiting, myalgia, and weight loss, with remission of self-limiting diarrhea in 3 to 4 days, followed by relapses lasting from 4 to 7 weeks. In patients with AIDS, symptoms may persist for as long as 12 weeks. Biliary disease has also been found in this group; acalculous cholecystitis was reported for HIV-positive and AIDS patients and resolved after initiation of treatment (35). These patients presented with right

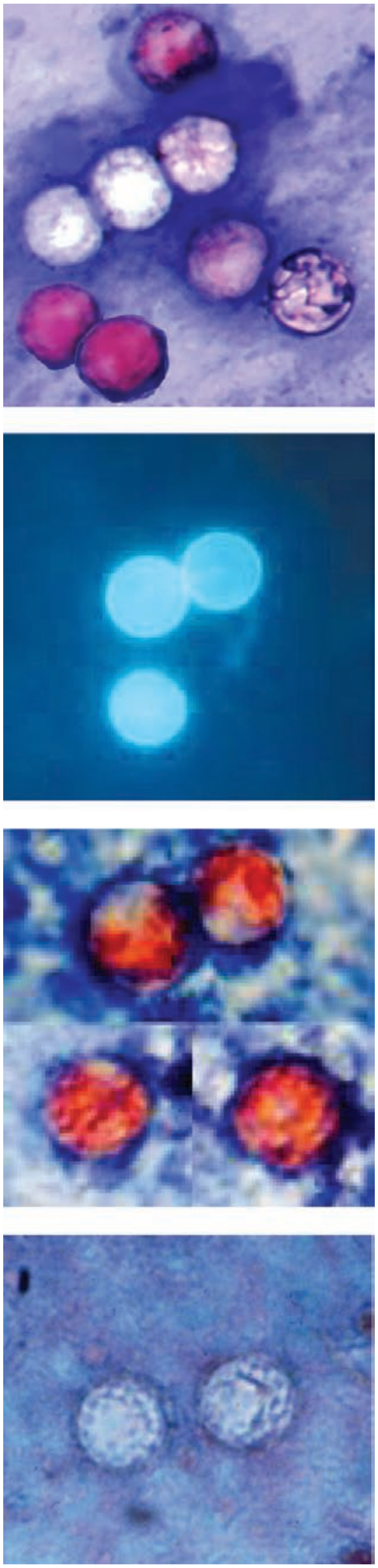


Figure 30.10 *Cyclospora cayetanensis*. (Top) Oocysts stained using the modified acid-fast stain (note the spherical shape; oocysts measure 8 to 10 µm; some oocysts do not stain, thus the organisms are said to be "modified acid-fast variable"). (Row 2) Autofluorescent oocysts using the appropriate filters for calcofluor white. (Row 3) Oocysts stained using the hot safranin method. (Bottom) Oocysts stained using the routine Wheatley's trichrome stain; note the oocysts do not stain well, but appear to be "ghost" cells. doi:10.1128/9781555819002.ch30.f10

TABLE 30.9 *Cyclospora cayetanensis*[a]

Information	Comments
Disease	
Haiti	Fewer infections in winter; summer temperatures may influence seasonality of infection; 34% in AIDS patients; exposure to water is a significant variable associated with infection
Guatemala	Children 5 times more likely to be infected than adults; more common in warmer months; high levels of contamination in rivers May–July; 18% in children 1 month to 1.5 yr old—most likely exposed from foods
Peru	2% in children 2 to 4 yr old; summer peak; 13% 1 to 9 yr old
Nepal	Oocysts identified in sewage water, vegetable washings; 11% in symptomatic travelers; 5% in children 6 to 60 months with diarrhea
Turkey	1.9% in hospital; higher in summer, 4.2% in another hospital study; 24.5% in immunocompromised patients with severe diarrhea (14.3% with *Cryptosporidium* as well)
Egypt	HIV-negative patient with weight loss, cough with purulent sputum, and dyspnea; suggested tuberculosis, *Cyclospora* confirmed by nested PCR; 2 cases on record; organism must be considered a new respiratory system pathogen; 5.6% with diarrhea, 2.3% without diarrhea
India	Important to test for intestinal parasites in HIV-positive patients
Reservoir hosts	
Worldwide	Pigs, cattle, horses, goats, dogs, cats, guinea pigs, chickens, ducks, turkeys, pigeons negative; humans may be the only natural host; controlled laboratory studies with freshwater clams (*Corbicula fluminea*) showed that 48 to 100% of the clams retained *Cyclospora* oocysts for up to 13 days
Disease risk factors	
Guatemala	U.S. infections linked to importation of Guatemalan raspberries; risk factors for workers include drinking water source, sewage drainage, proximity to chickens/other fowl; risk factors for children include contact with soil; drinking untreated water
Egypt	Sporulated and unsporulated oocysts found in tap water and lettuce heads; patients with Hodgkin's lymphoma on chemotherapy are at risk
Peru	Oocysts found in wastewater; may be link to oocysts on vegetables
North America	Linked to raspberries, mesclun, basil, snow peas; over 3,600 cases since 1995; tap water

[a]Adapted from references 32–35.

upper quadrant abdominal pain and elevated alkaline phosphatase levels. Diarrhea alternating with constipation has also been reported; this is not uncommon in a number of protozoal gastrointestinal infections. Clinical clues include unexplained prolonged diarrheal illness during the summer in any patient and in persons returning from tropical areas. The clinical presentation of patients infected with this organism is similar to that of patients infected with *Cryptosporidium* spp.

In clean wet mounts, the organisms are seen as nonrefractile spheres and are acid-fast variable with the modified acid-fast stain; those that are unstained appear as glassy, wrinkled spheres. Modified acid-fast stains stain the oocysts from light pink to deep red, and some contain granules or have a bubbly appearance. It is very important to be aware of these organisms when the modified acid-fast stain is used for *Cryptosporidium* spp. and other similar but larger structures (approximately twice the size of *Cryptosporidium* oocysts) are seen in the stained smear. It is important for laboratories to measure all acid-fast oocysts, particularly if they appear to be somewhat larger than those of *Cryptosporidium* spp. The oocysts autofluoresce green (1+ to 3+) (450 to 490 DM excitation filter) or blue (1+ to 3+) (365 DM excitation filter) under UV epifluorescence. It is strongly recommended that, during concentration (formalin/ethyl acetate) of stool specimens, the centrifugation time and speed should be 10 min at 500 × *g*. Molecular tests are becoming more widely available, as well.

Apparently, patients with *Cyclospora* infection do not respond to conventional antimicrobial therapies. Some patients have been treated symptomatically with antidiarrheal preparations and have obtained some relief; however, the disease appears to be self-limiting within a few weeks. Trimethoprim-sulfamethoxazole is currently the drug of choice; relief of symptoms has been seen in 1 to 3 days posttreatment. However, symptoms recur within 1 to 3 months posttreatment in over 40% of the patients. Although prophylaxis with trimethoprim-sulfamethoxazole is not generally recommended, it might be worth considering for an HIV-positive person visiting an area of endemic infection like Nepal during the summer, when the risk of infection tends to be the highest.

Published reports indicate that individuals of all ages, including immunocompetent and immunosuppressed individuals, can become infected. In Peru, infections with this organism have shown some seasonal variation, with peaks during April to June. This pattern is similar to that seen for *Cryptosporidium* infections in Peru. Preliminary data and extrapolation from what we currently know about cryptosporidiosis suggest that modes of transmission may be similar, particularly considering waterborne transmission.

Cystoisospora (*Isospora*) *belli*

An organism similar to *Cryptosporidium* that causes diarrhea in the compromised host is *Cystoisospora belli* (Tables 30.3 and 30.4; Fig. 30.11 and 30.12). Although *Cystoisospora* infections have been found in many areas of the world, certain tropical areas in South America appear to contain some well-defined locations of endemic infections. These organisms can infect both adults and children. Intestinal tract involvement and symptoms are generally transient unless the patient is immunocompromised (Tables 30.4 and 30.10). In a group of AIDS patients in Brazil, 2% were found to be positive for *C. belli* infection. In reviewing prevalence data for this and other parasites in this group, there did not appear to be any association between $CD4^+$ cell counts and any particular parasite. However, the data supported the value of using standard fecal examinations with this group of infected patients, even in the absence of diarrhea, since examinations can be easily performed, have a low cost, and can disclose treatable conditions (36). Since the introduction of highly active antiretroviral therapy (HAART), the prevalence of opportunistic infections has decreased dramatically; however, studies indicate that, unlike microsporidiosis and cryptosporidiosis, *Cystoisospora* prevalence has increased from 0.4 per 1,000 patients in the pre-HAART era to 4.4 per 1,000 patients in the HAART era (37).

Relapse tends to be common in both immunocompetent and immunosuppressed patients and is thought to be associated with extraintestinal stages (38). These unizoite tissue cysts in extraintestinal sites have been confirmed to be present in the liver, spleen, and mesenteric lymphoid tissues in AIDS patients (38, 39).

Cystoisospora infection is frequent in patients with AIDS in tropical areas. It has also been reported to occur in transplant recipients and in patients with other immunosuppressive diseases, such as lymphoblastic leukemia, adult T-cell leukemia, and Hodgkin's disease. Non-Hodgkin's lymphoma with chronic diarrhea due to *C. belli* has also been reported (40). *C. belli* can cause severe chronic diarrhea in patients with malignancies whose country of origin is in an area of endemic infection. Isosporiasis should be suspected in HIV-infected patients from tropical countries

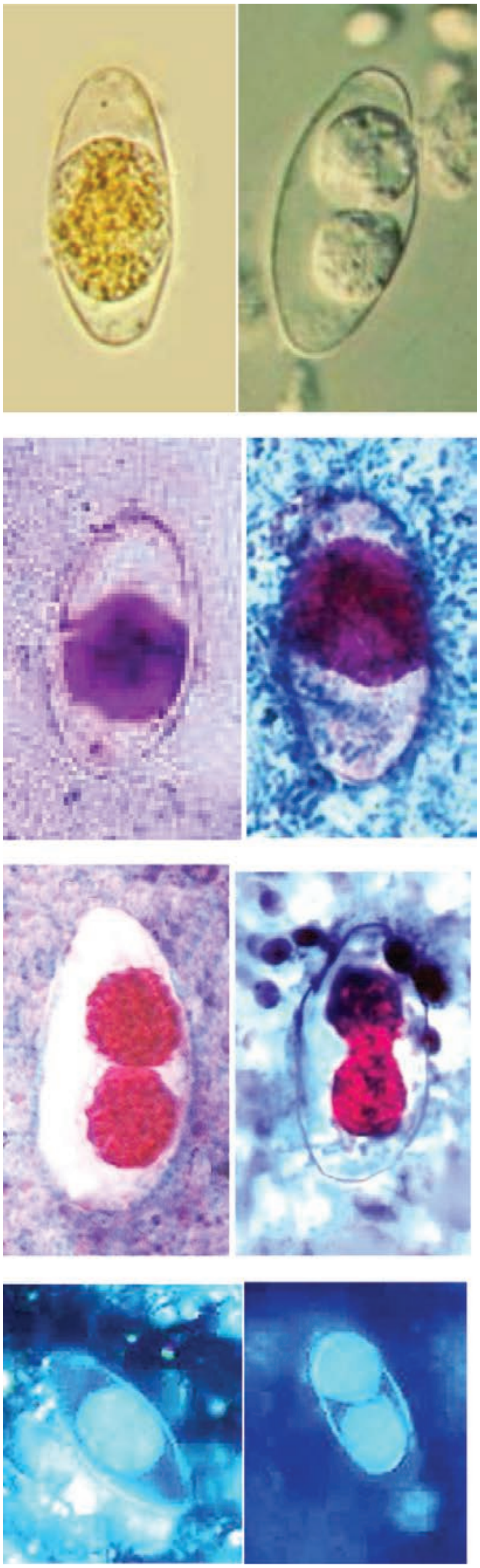


Figure 30.11 *Cystoisospora* (*Isospora*) *belli*. (Top) Wet mounts; immature oocyst (contains single sporoblast) on the left and mature oocyst on the right. (Row 2) Immature oocysts containing a single sporoblast, modified acid-fast stain. (Row 3) Mature oocysts containing two sporoblasts, modified acid-fast stain. (Bottom) Immature (one sporoblast) and mature (two sporoblast) oocysts in unstained wet mount viewed using UV fluorescence microscopy. (Bottom images courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch30.f11

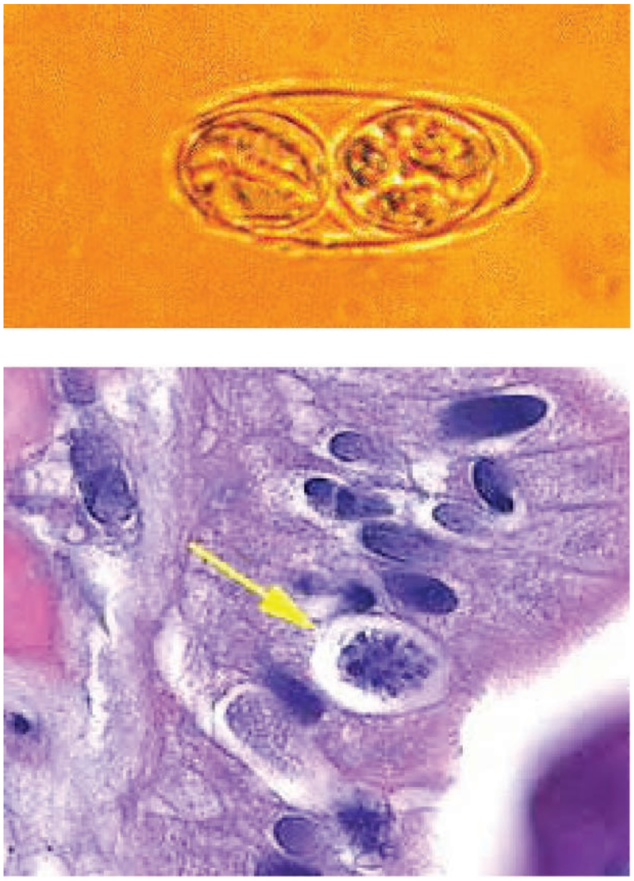


Figure 30.12 *Cystoisospora* (*Isospora*) *belli*. (Upper) Oocyst containing two sporocysts, each containing four sporozoites. (Lower) Developing stages in tissue, stained with hematoxylin and eosin (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch30.f12

presenting with diarrhea, weight loss, eosinophilia, and low $CD4^+$ cell counts.

Since *C. belli* infects both immunocompetent and immunocompromised adults and children, symptoms range from none to severe diarrhea. Persistent nonbloody diarrhea, similar to that seen with microsporidiosis or cryptosporidiosis, is the major symptom seen in immunocompromised individuals. Pathology with blunting of the villi, villous atrophy, and collagen deposition in the lamina propria is commonly seen. Extraintestinal infections have been detected in AIDS patients, revealing infections in lymph nodes, liver, spleen, and biliary system.

The life cycle of *C. belli* is generally considered to be similar to the life cycles of other coccidia; however, *C. belli* is thought to infect only humans. Oocysts are passed in the stool and are usually underdeveloped and noninfectious when passed. These oocysts are most often seen in wet preparation, such as the direct smear or the concentration sediment.

There has been much speculation over the years that many cases of *Cystoisospora* infection go undetected and that the incidence may be higher than that reported in the literature. However, it is also possible to have a positive biopsy specimen and not recover the oocysts in the stool because of the small numbers of organisms present. The oocyst wall in *C. belli* remains intact, and development can range from little differentiation to the presence of two well-defined sporocysts within the oocyst wall.

Sarcocystis spp.

Sarcocystis spp. require two hosts for completion of the life cycle. Two well-described species are *Sarcocystis* (*bovihominis*) *hominis* (cattle) and *Sarcocystis suihominis* (pigs). When raw or poorly cooked meat from infected animals is ingested by humans, gamogony occurs within the intestinal cells, with the production of sporocysts in the stool. In this case, humans who have ingested meat containing the mature sarcocysts serve as the definitive hosts. There have been reports of fever, severe diarrhea, abdominal pain, and weight loss in compromised hosts. Eosinophilic enteritis and ulcerative obstructive enterocolitis may be seen as occasional complications. In one group of Thai laborers, a 26.6% prevalence of *Sarcocystis* infection was seen. This high prevalence rate was indicative of the local habit of eating raw beef and pork; sarcocystosis could be a significant food-borne zoonotic infection in Thailand (41).

The sporocysts that are recovered in the stool are broadly oval, measuring 9 by 16 μm and containing four mature sporozoites and the residual body. Normally two sporocysts are contained within the oocyst (similar to *C. belli*); however, in *Sarcocystis* infections, the sporocysts are released from the oocyst and normally are seen singly (Fig. 30.13). They tend to be larger than *Cryptosporidium* oocysts, which contain four sporozoites, and so there should be no confusion between the two (42). Differentiation of encysted protozoan parasites in human feces is described in Table 30.11.

When humans serve as the accidental intermediate host, they accidentally ingest oocysts from other animal stool sources. The sarcocysts that develop in human muscle (schizogony) apparently do little if any harm (Fig. 30.14). There is essentially no inflammatory response to these stages in the muscle and no conclusive evidence of pathogenicity of the mature sarcocyst. However, symptoms have definitely been reported in some cases. A number of different morphologic types of skeletal and cardiac muscle sarcocysts have been recovered in humans; sarcocysts have also been found in muscles of the larynx, pharynx, and upper esophagus. Generally, histopathologic diagnoses are myositis with vasculitis and sometimes myonecrosis. No specific therapy is known for this type of infection, although

TABLE 30.10 *Cystoisospora* (*Isospora*) *belli*: parasite development and disease

Characteristic	Description	Comments
Infection source	Transmitted by infective oocysts in fecal-oral transmission	Excystation induced by body temperature with bile or bile salts and trypsin
Infective stage	Mature oocysts containing sporocysts with internal sporozoites; not infectious when passed	Oocyst wall is split, thus releasing sporozoites
Developmental stages	Zoites and meronts develop within epithelial cells; eventually merozoites form macrogametes and microgametes	Small intestine; in immunocompromised, parasite development occurs in biliary epithelial cells, as well; parasite cysts also seen in extraintestinal sites such as lymph nodes, liver, and spleen
Immunocompromised host	Recurrence of infection in AIDS patients following successful chemotherapy; recurrent diarrhea in AIDS patients receiving HAART	May be due to activation of cysts in extraintestinal sites
Cellular and molecular biology	Parasite material difficult to obtain; culture difficult, complete development difficult	Overall, little is known about the biology of this parasite
Clinical aspects	Infects both immunocompetent and immunocompromised patients; 54% have peripheral eosinophilia; vomiting, steatorrhea, headache, fever, malaise; symptoms in children can be severe	Most information comes from disease as seen in Chile; persistent nonbloody diarrhea like that seen with microsporidiosis and cryptosporidiosis
Immunology	CD4 T cells involved	Infection more severe in AIDS patients (low CD4 counts)
Pathology	Moderate to severe villous atrophy; collagen deposition in the lamina propria	Both sexual and asexual stages seen in epithelium, enclosed by parasitophorous vacuole
Diagnosis	Routine fecal ova and parasite examination; concentration important; some molecular tests available	Modified acid-fast stains helpful; auramine-rhodamine; unstained oocysts autofluorescent; intestinal biopsy (routine stains)
Epidemiology	Transmission most common in tropical zones (subequatorial Africa, Caribbean, parts of South America)	Prevalence in populations with high HIV infection is quite variable; percentages vary from 3% to over 20%; rarely seen in HIV-positive children
Treatment	Trimethoprim-sulfamethoxazole recommended	An approved drug, but considered investigational for this condition by the Food and Drug Administration

Adapted from references 36–40.

albendazole has been reported to be helpful (30). Characteristics of this infection can be seen in Table 30.12. In another four cases, the patients presented with lumps, pain in the limbs, or a discharging sinus. Histologic examination of biopsy specimens in all cases revealed characteristic cysts of *Sarcocystis* spp. Again, therapy is not specifically recommended; however, albendazole and steroids were also used in these cases.

In 2012, *Sarcocystis nesbitti* caused an acute relapsing febrile myositis in 89 of 92 participants who attended a retreat on Pangkor Island in Malaysia (43). A case was defined as a person who attended a specified retreat at a hotel on Pangkor Island, Malaysia, from 17 to 19 January 2012, and developed relevant clinical symptoms (fever, headache, myalgia and/or arthralgia) within 28 days upon return. Cases were subsequently defined as "definite sarcocystosis" if there was histological demonstration or nucleotide sequences of *Sarcocystis* spp. from muscle tissue. The remaining cases were defined as "probable sarcocystosis." Although sarcocysts have previously been noted as incidental findings in tongue muscles in autopsies, the presence of sarcocysts in these cases most probably represents a pathological process. Also, these results suggest that *S. nesbitti* has a very high attack rate (97%).

For infections in which humans serve as definitive hosts, prevention involves adequate cooking of beef and pork; for infections in which humans are intermediate hosts, preventive measures involve careful disposal of animal feces possibly containing the infective sporocysts. This may be impossible in wilderness areas, where wild animals may serve as reservoir hosts for many of the different types of organisms that have been grouped under the term "*Sarcocystis lindemanni*." However, evidence of multiple morphologically different cysts indicates that there are probably several species of *Sarcocystis* that cause human infections. Therefore, the name is no longer used.

Microsporidia

The microsporidia are obligate intracellular parasites that have been detected in a variety of animals, particularly invertebrates; the organisms found in humans tend to be quite small, ranging from 1.5 to 3 μm. Until recently, awareness and understanding of human infections have

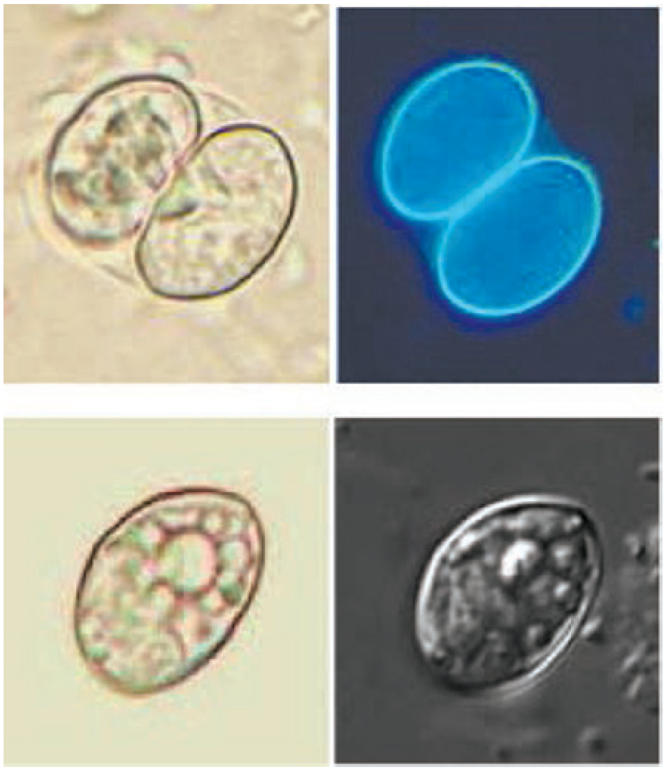


Figure 30.13 *Sarcocystis* sp. (Upper, left) Sporulated oocyst in unstained wet mount; (right) sporulated oocyst in a wet mount viewed under UV microscopy. (Lower, left) Sporocyst released from the oocyst; (right) sporocyst released from the oocyst, wet mount viewed under DIC microscopy. (Courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch30.f13

been marginal; only with increased understanding of AIDS within the immunosuppressed population has attention been focused on these organisms (Tables 30.3, 30.4, and 30.13; Fig. 30.15 and 30.16) (44–58). Limited availability of electron microscopy capability has also played a role in difficulties related to identification and classification of these infections.

Phylogenetic studies have confirmed that the microsporidia evolved from the fungi, being most closely related to the zygomycetes (44). Features shared with fungi include the presence of chitin and trehalose, similarities in cell cycles, and certain gene organizations. Microsporidia are now considered to be highly derived fungi that underwent genetic and functional losses, thus resulting in one of the smallest eukaryotic genomes known. However, at this point, clinical and diagnostic issues and responsibilities may remain with the parasitologists, and these organisms are maintained as a part of this parasitology text. We may be in a transition stage, similar to that seen with *Pneumocystis jiroveci* as it was moved from the parasites to the fungi in terms of classification status.

The organisms are characterized by having spores containing a polar tubule, which is an extrusion mechanism for injecting the infective spore contents into host cells. To date, several genera have been recognized in humans: the more common, *Encephalitozoon* and *Enterocytozoon*, and the less common, *Anncaliia*, *Pleistophora*, *Trachipleistophora*, *Vittaforma*, *Tubulinosema*, and *Microsporidium*, a catch-all genus for organisms that have not yet been classified (or may never be classified due to a lack of specimen). Classification criteria include spore size, configuration of the nuclei within the spores and developing forms, the number of polar tubule coils within the spore, and the relationship between the organism and host cell. Infection occurs with the introduction of infective sporoplasm through the polar tubule into the host cell. The microsporidia multiply extensively within the host cell cytoplasm; the life cycle includes repeated divisions by binary fission (merogony) or multiple fission (schizogony) and spore production (sporogony). Both merogony and sporogony can occur in the same cell at the same time. During sporogony, a thick spore wall is formed, providing environmental protection for this infectious stage of the parasite. An example of infection potential is illustrated by *Enterocytozoon bieneusi*, an intestinal pathogen. The spores are released into the intestinal lumen and are passed in the stool. These spores are environmentally resistant and can then be ingested by other hosts.

Encephalitozoon

Both *Encephalitozoon cuniculi* and *Encephalitozoon hellem* have been isolated from human infections, the first species from the CNS and the second from the eye. The first case occurred in a 9-year-old Japanese boy who presented with neurologic symptoms such as convulsions, vomiting, headaches, fever, and periods of unconsciousness (44). The spores were recovered in the CSF and in urine. This particular patient was treated with sulfisoxazole and recovered. Another case of *E. cuniculi* infection was in a 2-year-old child with a similar neurologic illness; the spores were recovered from the urine. A recent case of keratoconjunctivitis and chronic sinusitis due to infection with *E. cuniculi* has been found in an AIDS patient; the diagnosis was confirmed by electron microscopy (44). Several eye infections with *E. hellem* have been found in AIDS patients, including the first reported case of *E. hellem* infecting not only sinuses and conjunctivae but also the nasal epithelium. In the first case of disseminated *E. hellem* infection in an AIDS patient, a complete autopsy revealed organisms in the eyes, urinary tract, and respiratory tract. The presence of numerous organisms within the lining epithelium of almost the entire length of the tracheobronchial tree was very suggestive of respiratory acquisition. It is also interesting that although *E. cuniculi* and *E. hellem*

TABLE 30.11 Encysted pathogenic protozoan parasites seen in human feces

Protozoan parasite	Size (µm)	Shape	Features
Sarcocystis hominis and *S. suihominis*			Excreted sporulated (immediately infectious)
Oocysts	15–19 by 15–20	Spherical	Contain 2 sporocysts; often seen as single sporocysts released from the oocyst
Sporocysts	15–19 by 8–10	Oval	Contain 4 sporozoites
Cystoisospora (*Isospora*) *belli*	20–33 by 10–19	Ovoid with tapered ends	Excreted unsporulated (not immediately infectious)
Cyclospora cayetanensis	7.7–10	Spherical	Excreted unsporulated (not immediately infectious)
Cryptosporidium hominis and *C. parvum*	4–6	Nearly spherical	Excreted sporulated (immediately infectious); some internal sporozoites difficult to see
Giardia lamblia	11–14	Spherical to ovoid to ellipsoid	4 nuclei, curved median bodies, linear axonemes (immediately infectious)
Dientamoeba fragilis	4–5	Spherical	Distinct cyst wall; cysts quite rare in clinical specimens
Entamoeba histolytica and *E. dispar* (*E. histolytica/E. dispar* group)	10–15	Spherical	Precyst generally contains single nucleus (karyosome with peripheral nuclear chromatin); mature cyst contains 4 nuclei and possible chromatoidal bars (with round, smooth ends) (mature cyst immediately infectious)
Blastocystis spp. (currently discussed with the protozoa)	6–40	Spherical to somewhat oval	Central body form with nuclei around the central body area (immediately infectious as far as known)
Balantidium coli	50–70	Spherical to oval	Large macronucleus, thick wall (infectious)

differ biochemically and immunologically, their fine structure and development are indistinguishable. Some of the eye infections have also suggested a topical route rather than dissemination.

Microsporidiosis is now recognized as an emerging transplant-associated disease and should be considered in febrile transplant recipients when tests for routinely encountered agents are unrevealing. Donor-derived disease is critical to assess when multiple recipients from a common donor are ill (45).

Anncaliia (*Brachiola, Nosema*) spp.

Anncaliia connori (formerly referred to as *Brachiola connori* or *Nosema connori*) was identified in human tissues in a 4-month-old infant with combined immunodeficiency disease who had contracted a disseminated, fatal infection. Parasites were found in the myocardium, diaphragm, arterial walls, kidney tubules, adrenal cortex, liver, and lungs. Concurrently, this patient also had *P. jiroveci* pneumonia. *A. algerae* (formerly *Brachiola algerae* or *Nosema algerae*) is apparently transmitted by a mosquito. Infections have been found in the eye and muscle, possibly from a disseminated case. Both immunocompromised (HIV-negative, but receiving immunosuppressive therapy for rheumatoid arthritis) and immunocompetent patients have become infected. *Anncaliia vesicularum* has also been associated with AIDS and myositis. Muscle tissue obtained at biopsy and examined by light and electron microscopy revealed organisms developing in direct contact with muscle cell cytoplasm and fibers. Apparently, no other tissue types were infected. Clinical and histologic clearing of the infection was achieved after treatment with albendazole and itraconazole.

Pleistophora and *Trachipleistophora* spp.

Microsporidia in the genera *Pleistophora* and *Trachipleistophora* have rarely been identified in humans. However, when *Pleistophora* was found, atrophic and degenerating muscle fibers were full of spores which were seen in clusters of about 12 organisms, each cluster enclosed by an enveloping membrane, the pansporoblastic membrane. The disease was characterized by a 7-month history of progressive generalized muscle weakness and contractions, in addition to fever, generalized lymphadenopathy, and an 18-kg weight loss. Although the patient was diagnosed as having AIDS, the presence of HIV was never demonstrated. Specimens from early biopsies demonstrated atrophic and degenerating muscle fibers; specimens from later biopsies showed fibrosis and scarring. *Trachipleistophora hominis* causes severe myositis and sinusitis and was first found in an AIDS patient. To date, no natural hosts other than humans have been identified; however, when inoculated intraperitoneally into athymic mice, the organisms infect the skeletal muscle, as seen in human infections. *T. antropophtera* is the most recently described microsporidian in humans and has been found in brain tissue of two AIDS patients at autopsy; organisms were also found in the kidneys and heart.

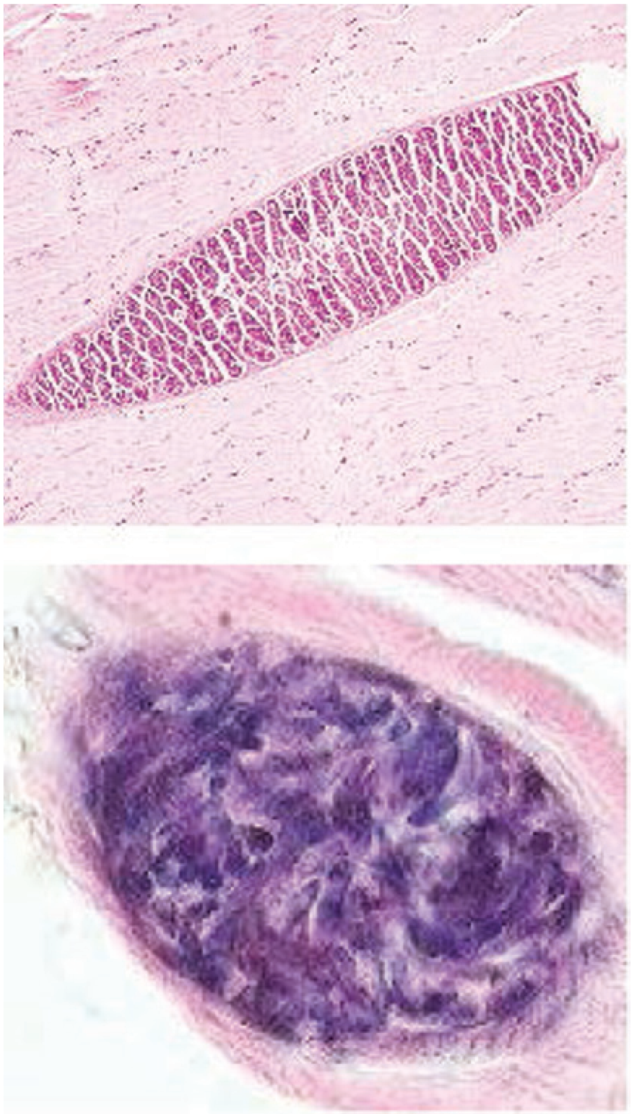


Figure 30.14 *Sarcocystis* sp. in human muscle tissue (note the bradyzoites contained within the sarcocyst, visible in the lower image). doi:10.1128/9781555819002.ch30.f14

Tubulinosema acridophagus

Microsporidiosis subsequently developed in a stem cell transplant patient with multiple myeloma, probably linked to immunosuppressive steroid use. The microsporidian was identified as *Tubulinosema acridophagus*, a new human pathogen (54). Electron microscopy studies revealed spores in skin and peritoneal fluid that were consistent with the original identification. The spores contained the typical polar tubule with 11 to 14 coils. After extensive tissue examination, spores were confirmed in skin, liver, peritoneum, lung, and several eye tissues. *T. acridophagus* species has also been identified from muscle in a patient with non-Hodgkin's lymphoma (55).

Enterocytozoon bieneusi

Although currently there are several genera of microsporidia, one of which is the catchall genus *Microsporidium*, human infections with some genera are more common than with others. A number of infections with *Enterocytozoon bieneusi* have been found in AIDS patients. Chronic intractable diarrhea, fever, malaise, and weight loss are symptoms of *E. bieneusi* infections and are similar to those seen with cryptosporidiosis or isosporiasis. These patients have four to eight watery, nonbloody stools per day, which can be accompanied by nausea and anorexia. There may be dehydration with mild hypokalemia and hypomagnesia, as well as D-xylose and fat malabsorption. The patients tend to be severely immunodeficient, with a CD4 count always below 200/mm^3 and often below 100/mm^3. *E. bieneusi* infection has also been implicated in AIDS-related sclerosing cholangitis.

Dual infections with *E. bieneusi* and *Encephalitozoon intestinalis* have been reported. *E. intestinalis* infects primarily small intestinal enterocytes, but infection does not remain confined to epithelial cells. *E. intestinalis* is also found in lamina propria macrophages, fibroblasts, and endothelial cells. Dissemination to the kidneys, lower airways, and biliary tract appears to occur via infected macrophages. Fortunately, these infections tend to respond to therapy with albendazole, unlike the infections with *E. bieneusi*.

A number of techniques for recovery and identification of microsporidia in clinical specimens are available. Although the organisms can be identified in routine histologic preparations, they do not tend to stain predictably. Occasionally, the spores take on a refractile gold appearance in formalin-fixed, paraffin-embedded, routine hematoxylin-and-eosin-stained sections. Some of the difficulty may be attributed to the use of formalin; alternative fixatives are currently being tried. Spores are occasionally seen very well by using the PAS stain, the methenamine silver stain, tissue Gram stains, or acid-fast stains. The spore has a small, PAS-positive posterior body, the spore coat stains with silver, and the spores are acid-fast variable. There is also evidence to indicate that specimens from plastic-embedded tissues are seen more easily, regardless of the fixative used. Tissue examination by electron microscopy is still considered the best approach; however, this option is not available to all laboratories and is not that sensitive.

Techniques that do not require tissue embedding are also popular. Touch preparations of fresh biopsy material that are air dried, methanol fixed, and Giemsa stained have been used; however, screening must be performed using the 100× oil immersion objective. Another study, involving cytocentrifugation followed by Giemsa staining, found that 27% of 55 AIDS patients with chronic diarrhea were

TABLE 30.12 *Sarcocystis*: parasite development and disease[a]

Characteristic	Infection in muscle:human as accidental intermediate host	Infection in intestine:human as definitive host
Infection source	Food or water contaminated with feces from unknown carnivore or omnivore	Raw or undercooked meat
Infective stage	Oocyst or free sporocysts	Sarcocyst containing bradyzoites
Developmental stages	Intravascular schizonts (not seen); intramuscular sarcocysts	Sexual stages in lamina propria; oocysts passed in feces
Incubation period from ingestion to symptoms	Weeks to months; can last months to years	3–6 h, lasting 36 h
Symptoms	Musculoskeletal pain, fever, fleeting pruritic rash, transient lymphadenopathy, subcutaneous nodules associated with eosinophilia, cardiomyopathy, and bronchospasm	Nausea, loss of appetite, vomiting, stomachache, bloat, diarrhea, dyspnea, and tachycardia
Diagnosis	Biopsy specimen containing sarcocyst; antibodies to bradyzoites (routine histology)	Oocysts or sporocysts in feces, beginning 5–12 days after ingestion (routine ova and parasite examination, particularly concentration)
Therapy (none approved)	Albendazole, anti-inflammatories	None

[a] Adapted from reference 42.

positive for *E. bieneusi*; all results were confirmed by electron microscopy.

Routine staining of clinical specimens (stool, urine, eye specimens) can be performed using a modified trichrome stain in which the chromotrope 2R component added to the stain is 10 times the concentration normally used in the routine trichrome stain for stool. The stool preparations must be *very* thin, the staining time is 90 min, and the slide must be examined at ×1,000 (or higher) magnification. Unfortunately, there are many objects within stool material that are oval, stain pinkish with trichrome, and measure approximately 1.5 to 3 μm. If this stain is used for the identification of microsporidia in stool, positive control material should be available for comparison. The newest approach for the identification of spores in clinical specimens uses antisera in an IFA procedure. Fluorescing microsporidial spores are distinguished by a darker cell wall and by internal visualization of the polar tubule as diagonal lines or cross lines within the cell. In another study with this same antiserum, 9 (33%) of 27 patients diagnosed as having cryptosporidiosis (8 AIDS patients and 1 non-AIDS but immunodeficient patient) were found to have *E. bieneusi* in the stool. Although there is some cross-reactivity with bacteria, this technique offers a more sensitive approach than routine staining methods currently available for the examination of stool specimens. As clinicians begin to suspect these infections and laboratorians become more familiar with the diagnostic methods, the number of positive patients, particularly those who are immunocompromised, may increase dramatically.

Although *E. bieneusi* appears to be the most common human microsporidian, serologic evidence is based primarily on results for *Encephalitozoon cuniculi* as the parasite antigen. Whether these surveys reveal actual infections with this organism or whether there is cross-reactivity with other microsporidians is unknown. However, the data strongly suggest that latent infections with microsporidia occur in a number of groups, ranging from patients with other infections such as tuberculosis, typhoid fever, leprosy, malaria, schistosomiasis, toxoplasmosis, Chagas' disease, and toxocariasis, to normal individuals and a group of homosexual men (56). Serologic evidence of human infection with the microsporidia has been reviewed elsewhere (56, 57). Microsporidian infections in immunocompetent patients may result in a self-cure following mild symptoms over a short time frame, a situation similar to that seen with both cryptosporidiosis and cystoisosporiasis. As awareness of this infection increases and more sensitive diagnostic techniques are developed, we may find that these infections are not uncommon in immunocompetent hosts.

The vast majority of human cases of microsporidiosis have been reported during the past 20 years in patients with HIV/AIDS, while only relatively rare cases have been described in immunocompetent individuals. However, microsporidial infections are being increasingly found in patients following solid-organ transplantation, where the main symptom has been diarrhea. The first case of pulmonary microsporidial infection in an allogeneic bone marrow transplant recipient in the United States and only the second case in the world has been reported (58).

Leishmania spp.

Most *Leishmania* and HIV coinfection cases reported to the World Health Organization come from Southern Europe. Up to 2001, nearly 2,000 cases of coinfection were

TABLE 30.13 Microsporidia: AIDS patients

Findings in AIDS patients	Comments[a]
Intestinal microsporidiosis	High proportion of patients remained symptomatic after 6 mo (54.8% with persistent diarrhea, 51.2% with weight loss); predictors include high HIV RNA viral load and no initiation of protease inhibitor therapy
AIDS patients in Venezuela	The samples from 42 patients were analyzed by separate PCRs to identify *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi*. Microsporidia species showed 36% prevalence: 10 patients had *Encephalitozoon intestinalis*, 4 had *Enterocytozoon bieneusi*, and 4 had both species. An inverse and statistically significant relationship between the CD4 count and the presence of microsporidia in the fecal sample was also found. The high prevalence of microsporidia species observed in the HIV patients studied, with a predominance of *E. intestinalis*, is remarkable.
Children in Spain	1.2% overall and 2% of those with diarrhea (n = 83) had microsporidiosis; different data from those previously reported for adults (13.9%) in the same area, although adult group showed more severely depressed CD4 lymphocyte counts than children did
Children in Uganda	Some 81% of HIV-infected children with persistent diarrhea excreted *Cryptosporidium*, *Enterocytozoon bieneusi*, or both organisms, compared with only 10% of children with persistent diarrhea testing negative for HIV; 74% of isolates were *C. hominis* (the anthroponotic species), 17% were *C. parvum* (the zoonotic species), and 8% were a mixture of the 2 or others
Patients with or without diarrhea in Germany	Prevalence of intestinal microsporidiosis varies from 7 to 50%; most common enteropathogen in 18/50 patients (36%) with diarrhea and 2/47 (4.3%) without diarrhea; microsporidia present in 60% of patients with chronic diarrhea and 5.9% of patients with acute diarrhea (*E. bieneusi* in 18, *E. intestinalis* in 2); prevalence in this group one of the highest in the world
Mucosal abnormalities	Mechanisms appear to be malabsorption caused by reduction of absorptive mucosal surface and impairment of enterocyte function
Epidemiology	
Waterborne outbreak	Of 338 individuals, 261 had HIV, 16 were transplant recipients, and 61 had other sources of immunosuppression; disease appears to be endemic in HIV-positive persons; 200 people involved in waterborne outbreak; no evidence of fecal contamination of water; outbreak ended several months before antiprotease era
Risk factors	Intestinal microsporidiosis in persons with HIV infection and ≤200 CD4 cells/mm^3 is associated with male homosexuality and swimming in pools, suggesting fecal-oral transmission, including sexual and waterborne routes
New disease manifestation	
Nodular skin disease	36-yr-old woman presented with late-stage AIDS and disseminated, nodular cutaneous lesions and underlying osteomyelitis; disseminated microsporidial infection with *Encephalitozoon*-like species diagnosed by EM; long-term clindamycin therapy cured cutaneous infection; response to clindamycin merits further study
Current clinical syndromes	Enteropathy, keratoconjunctivitis, sinusitis, tracheobronchitis, encephalitis, interstitial nephritis, hepatitis, cholecystitis, osteomyelitis, myositis; *E. bieneusi* is the most common cause of intestinal disease (can also disseminate); *E.* (*Septata*) *intestinalis* is associated with disseminated and intestinal disease
Modification of clinical course	Of 37 AIDS patients with *E. bieneusi* diarrhea, 15 showed clearance (40.5%); peripheral blood CD4 cell count of ≤100/mm^3, use of two or more antiretroviral medications, and use of protease inhibitor statistically associated with time to clearance of parasite; albendazole not associated with parasite eradication
Corneal infections	Microsporidia cause keratoconjunctivitis, corneal stromal keratitis, scleritis, and endophthalmitis; the corneal picture resembles herpes simplex virus stromal keratitis or fungal keratitis cases, and is characterized by deep stromal infiltrates with overlying and surrounding stromal edema and keratic precipitates; the diagnosis of microsporidial infection is confirmed by a microscopic examination of smears from patients with ulcerative keratitis or by a histopathological examination of corneal tissues
Zoonotic *Enterocytozoon bieneusi* genotypes in HIV-positive patients	*E. bieneusi* genotypes were detected and differentiated by PCR and DNA sequencing. The infection rates were 5.7% and 4.2% for *E. bieneusi* in HIV-positive and HIV-negative participants, respectively. Prevalent *E. bieneusi* genotypes detected, including EbpC (39), D (12), and type IV (7), which were also potentially zoonotic

[a] Adapted from references 46–53. EM, electron microscopy.

identified, of which 90% were from Spain, Italy, France, and Portugal. However, these figures are probably underreported because they do not account for the large number of cases in Africa and Asia that are missed due to a lack of diagnostic facilities and poor reporting systems. Most cases of coinfection in the Americas are reported in Brazil. In some areas of Africa, the number of coinfection cases has increased dramatically due to population migration

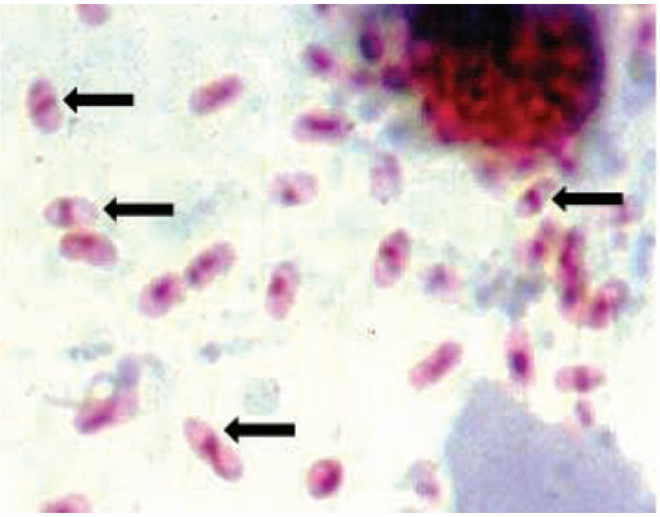


Figure 30.15 Microsporidia. Microsporidial spores seen in nasopharyngeal aspirate from AIDS patient; note the horizontal or diagonal lines (arrows) representing the polar tubules within the spores, stained with Ryan modified trichrome stain. doi:10.1128/9781555819002.ch30.f15

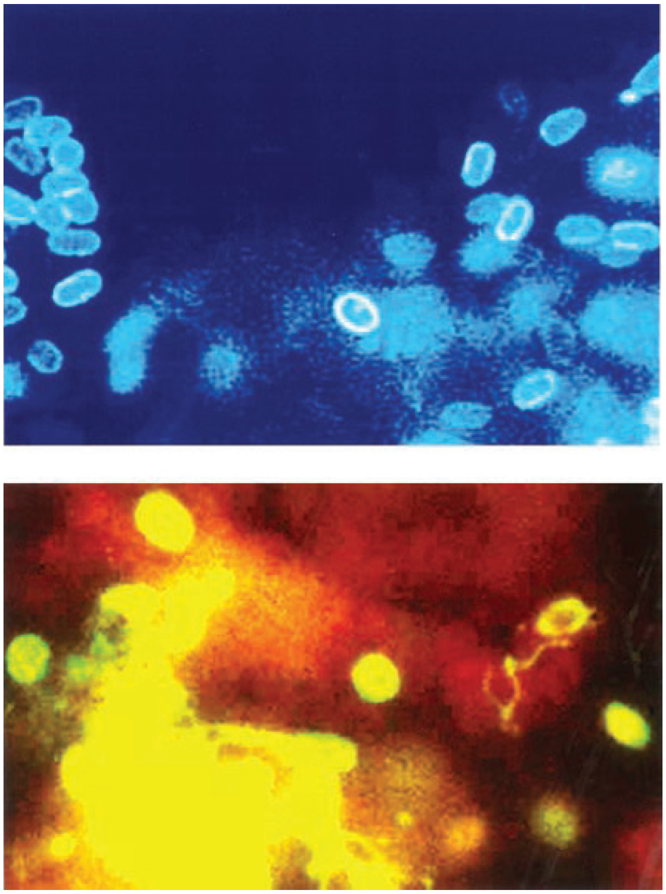


Figure 30.16 Microsporidial spores. (Upper) *Encephalitozoon intestinalis* stained with calcofluor white. (Lower) Monoclonal-antibody-based immunofluorescence identification of *Encephalitozoon hellem* (Courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch30.f16

and wars. In northwest Ethiopia, up to 30% of all visceral leishmaniasis patients are also infected with HIV. In Asia, coinfections are increasingly being reported in India, which also has the highest global burden of leishmaniasis and a high rate of resistance to antimonial drugs (59–67). Visceral leishmaniasis (VL) continues to increase in frequency in patients who are HIV positive. The majority of cases of leishmaniasis in AIDS patients have been seen in France, Spain, Italy, and Portugal. Like other opportunistic infections, subclinical VL can be found at any stage of HIV-1 infection, but symptomatic cases appear mainly when severe immunosuppression is present. HIV-*Leishmania* coinfection is being seen more and more frequently in the Mediterranean basin (Tables 30.14 and 30.15; Fig. 30.17).

This finding strongly suggests that individuals infected with *Leishmania* spp. without symptoms will express symptoms of leishmaniasis if they become immunosuppressed. Based on the patient's general health status and prior exposure to the infection, leishmaniasis may be seen as an early opportunistic infection or may be seen as a complication late in the course of AIDS. Clinical signs of VL usually occur in the later stages of AIDS. The majority of AIDS patients present with the classic picture of VL; however, asymptomatic cutaneous, mucocutaneous, diffuse cutaneous, and post-kala azar dermal leishmaniasis (usually caused by *L. infantum*) can be seen. Cutaneous lesions in VL are being detected increasingly frequently in patients with HIV infection, and their significance is still somewhat unclear. Lesions often do not present a uniform or specific appearance and have been seen as erythematous papules and hypopigmented macules on the dorsa of the hands, feet, and elbows; small subcutaneous nodules on the thighs; and erythematoviolaceous, scaly plaques on the face. Also, the digestive and respiratory tracts are often parasitized, as well as the pleura and peritoneum. Lesions can also be coinfected with other organisms, such as *Mycobacterium avium-intracellulare*.

The HIV/AIDS pandemic has modified the natural history of leishmaniasis. HIV infection increases the risk of developing VL by 100 to 2,320 times in areas of endemicity, reduces the likelihood of a therapeutic response, and greatly increases the probability of relapse (65, 66). At the same time, VL promotes the clinical progression of HIV disease. Both diseases exert a synergistic detrimental effect on the cellular immune response because they target similar immune cells. VL occurring during HIV infection cannot be cured, even after extensive antileishmanial treatment. The likelihood of a relapse occurring is dependent on the

TABLE 30.14 *Leishmania* infections in immunocompromised patients[a]

Underlying condition	Disease manifestation	Comments
AIDS	VL (southern Spain)	HIV-1 carriers (291) underwent bone marrow aspiration (regardless of symptoms); 32 (11%) showed VL and 13 of these (41%) had subclinical cases; female sex negatively associated with VL; i.v. drug use high, but not independent association; high proportion of cases subclinical; symptomatic cases evident when deep immunosuppression is present
AIDS	VL (Mediterranean area)	*Leishmania*-HIV coinfection common in Mediterranean area; most common species is *L. infantum*; most frequent if CD4 count is <200/mm^3 and in parenteral drug addicts; chronic development with relapses is frequent; most effective diagnostic method is bone marrow culture (NNN); low sensitivity for serologies; PCR useful in asymptomatic cases, for therapeutic control, and in relapses; treatment similar to that in immunocompetent patients; generally necessary to use secondary prophylaxis; mortality rate is high (~25%)
AIDS	VL and possible dissemination	WHO estimates that 2–9 in 100 HIV patients in Africa and Asia will develop *Leishmania*-HIV coinfection; VL most common; unusual locations suggest multiorgan spreading in absence of host immune response; high incidence of recurrence; follow-up required; clear picture of prophylaxis not yet developed
AIDS	VL	Since 1990, 1,616 cases of coinfection reported, mainly from southern Europe (Spain, southern France, Italy); coinfected patients mainly young adults, i.v. drug users; 272 isolates showed 18 different zymodemes, 10 of which were found only during HIV coinfection; new foci emerging (Brazil and East Africa)
AIDS	VL (and mucocutaneous disease in areas where *L. donovani* is not endemic)	15 AIDS patients in Germany, all of whom were severely immunocompromised; 1 was diagnosed at autopsy; 1 mucocutaneous case was diagnosed by nasal biopsy; others were diagnosed by bone marrow (13/13), liver (3/3), and gastrointestinal mucosa (4/4) biopsies; serology positive in only 6/13; VL must be ruled out in every patient with fever and/or pancytopenia and appropriate travel history
AIDS	Case mimicking VL	HIV-positive patient presented with picture of VL coinfection; bone marrow biopsy performed on admission; amastigote forms seen in bone marrow, and organisms grew in culture as promastigotes; molecular analysis revealed that the organisms were not *Leishmania*, but were *Leptomonas pulexsimulantis*, a trypanosomatid found in dog fleas; thus, opportunistic infection with an insect trypanosomatid was suspected and confirmed
Immunosuppressive drugs for organ transplantation or cancer	VL	Immunosuppressive therapy results in reactivation of asymptomatic VL or facilitates new infections
AIDS	CL (Brazil)	IRIS has been found in AIDS patients since the introduction of HAART; syndrome is characterized by clinical manifestations of opportunistic infections when signs of immune reconstitution are observed during therapy; leishmaniasis, suggestive of HAART-induced IRIS, was seen in 2 patients with AIDS; after beginning HAART, 1 patient presented with disseminated CL lesions, whereas the other patient's preexisting lesions worsened and became more extensive; however, their CD4$^+$ T-cell counts were increasing and viral loads were decreasing significantly; the lesions healed with anti-*Leishmania* therapy
AIDS	CL (French Guiana)	In French Guiana, CL in moderately immunosuppressed HIV-infected subjects (CD4$^+$ T-cell count, >200/mm^3) is characterized by a higher rate of recurrence or reinfection and is more difficult to treat than that in HIV-negative subjects
AIDS	CL (Italy)	Patient was HIV infected, an i.v. drug user, with an unusual disseminated CL after an initial visceral disease and after a 13-month maintenance treatment with liposomal amphotericin; severe concurrent immunosuppression probably played a role in leading to this atypical cutaneous form, characterized by diffuse, nonulcerated, nonscabby maculopapular lesions

[a] Adapted from references 59–67. CL, cutaneous leishmaniasis; HAART, highly active antiretroviral therapy; IRIS, immune reconstitution inflammatory syndrome; i.v., intravenous; NNN, Novy-MacNeal-Nicolle medium; VL, visceral leishmaniasis; WHO, World Health Organization.

level of CD4$^+$ cells in the patient. For patients coinfected with HIV-1 and *Leishmania*, the increase in cytokine production induced by the parasite will probably affect virus production not only in macrophages but also in HIV-1-infected CD4$^+$ T cells.

CD4 T-lymphocyte counts are usually less than 50/mm^3 and are almost always less than 200/mm^3. Many physicians think that VL does not cause severe symptoms in these patients but is just one of several opportunistic infections that may coinfect AIDS patients. However, VL

TABLE 30.15 Epidemiological information on *Leishmania*-HIV coinfection[a]

Area	Specific countries	Comments
Southern Europe	France, Italy, Portugal, Spain	Largest number of cases in Spain; Spain has 5–7 times more new infections among IV drug users; 75% patients live in coastal urban areas; spread from urban to rural areas; decrease by end of 1990s due to HAART; >90% of cases are relapses rather than reinfections; 94% of patients with *Leishmania*-HIV coinfection suffered from VL, and 4% had CL
South Asia	Bangladesh, India, Nepal	Currently this area is the main focus of VL in the world, accounting for 67% of total leishmaniasis; many cases underreported; 2 to 3.1 million HIV cases in India; positive rates of coinfection range from 1.5 to 6.3%; no data available from Bangladesh
Americas	Brazil	Epicenter of the HIV/AIDS epidemic in South America; from 2001 to 2005, 16,210 cases of VL were reported in Brazil, and 315 (2%) patients were coinfected with HIV; of 150,000 CL cases reported from 2001 to 2005, 150 (0.1%) patients had HIV coinfection. MCL is frequently observed in HIV-coinfected patients in Brazil (43%), a clinical pattern rarely seen in southern Europe; in Brazil, 43%, 37%, and 20% of the burden of coinfection are due to MCL, VL, and CL, respectively
Sub-Saharan Africa	Burkina Faso	HIV seroprevalence is 2%; CL reported from many areas; major increase in cases since 2000; many unusual forms of CL found (lepromatous/diffuse, ulcerative, infiltrative, papulonodular, psoriasis-like, Kaposi's sarcoma-like, keloid, histoid); rapid relapses seen after therapy in HIV coinfected patients
	Ethiopia	Incomplete information (no current surveillance system); VL endemicity dates back to the 1940s; increase in coinfection from 18.5% in 1998–1999 to 40% in 2006
	Kenya, Uganda, Somalia	Data very limited; risk of coinfection is increasing; no coinfection yet reported in Somalia
	Sudan	15,000–20,000 VL cases; prevalence of AIDS 1.6%; HIV/AIDS cases estimated at 350,000; number on the increase; coinfection now recognized
Overall		1,037 strains of *Leishmania* isolated and identified from HIV+ patients; majority from southwest Europe

[a]Adapted from references 65 and 66. CL, cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; VL, visceral leishmaniasis.

can manifest atypical aspects in HIV-positive patients depending on the degree of immunosuppression, and it should be listed among AIDS-defining conditions. The most common symptoms are fever, splenomegaly, hepatomegaly, and pancytopenia.

Data from Spain provide some interesting outcome figures regarding *Leishmania* infections in persons with or without HIV infections. In spite of a good initial response to treatment for VL, 60.6% of the patients had relapsed by the end of 1 year. Mortality from the first episode was 18.5%, and 24% died in the first month after diagnosis of any VL episode. The mean survival of the 29 patients who died was 10.27 months. Survival in patients with and without AIDS at the time of the first episode of VL was compared at 30 months and found to be 30.5 and 53.7%, respectively. A diagnosis of AIDS, at the time of the first episode of VL, and thrombocytopenia were the only risk factors found to be related to survival. Certainly in AIDS patients, VL is a recurrent disease that is highly prevalent and whose clinical course is modified by HIV. VL is a very prevalent disease among HIV-1-infected patients in southern Spain, with a high proportion of cases being subclinical. Symptomatic cases appear mainly when severe immunosuppression is present. There is also an association between VL and the male sex and intravenous drug use. In HIV-infected patients, VL occurs in late stages of HIV disease and often has a relapsing course. Secondary prophylaxis reduces the risk of relapse.

In HIV-1-infected patients with active VL, the sensitivity of a peripheral blood smear is about 50% because of the high parasitemia in these patients. However, in HIV-1-infected patients with subclinical VL, the sensitivity of a routine blood smear is <10%. Unfortunately, in these patients serology and *Leishmania* skin tests have low sensitivities and are little help. Cultures have proven to be effective in diagnosis of VL in patients who are coinfected with HIV-1.

A clinical diagnosis of Old World VL is often made based on clinical findings and local epidemiologic factors. However, confirmation of the diagnosis requires demonstration of the amastigotes in tissues or clinical specimens (blood, buffy coat preparations) or the promastigotes in culture. Detection of parasite genetic material or antigen detection is also sufficient for confirmation. Leishmaniasis should be suspected in individuals who have resided in or traveled to areas where the disease is endemic. The diagnosis would be supported by findings of remittent fevers, hypergammaglobulinemia with anemia, circulating immune complexes, rheumatoid factors, weight loss, leukemia, and splenomegaly. The differential diagnosis of late-stage VL is limited to hematologic and lymphatic malignancies. The diagnosis of early disease is more difficult, and

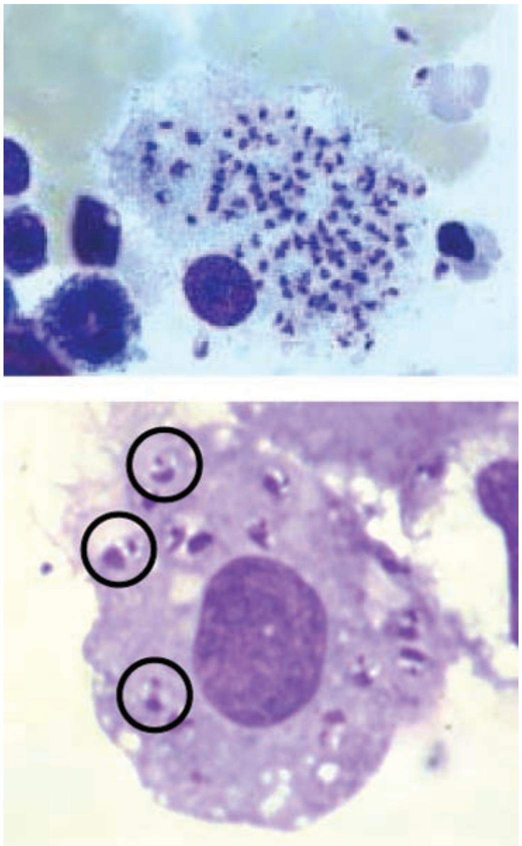


Figure 30.17 *Leishmania donovani* in bone marrow; specimen stained using Giemsa stain. Note the individual amastigotes with the larger nucleus and bar-shaped kinetoplast (circles). doi:10.1128/9781555819002.ch30.f17

the differential would include malaria, brucellosis, enteric fevers, bacterial endocarditis, generalized histoplasmosis, chronic myelocytic leukemia, Hodgkin's disease and other lymphomas, sarcoidosis, hepatic cirrhosis, and tuberculosis. Also, patients with multiple myeloma and Waldenström's macroglobulinemia have monoclonal hypergammaglobulinemia. Mixed cryoglobulinemia secondary to VL has also been reported.

Although the usual route of transmission is through the bite of infected sand flies, parasites can be transmitted in other ways including blood transfusion, sexual contact, congenital transmission, occupational exposure, and shared needles. Apparently, *L. infantum* can circulate intermittently and at low density in the blood of healthy seropositive individuals, who appear to be asymptomatic carriers; this certainly has implications for the safety of the blood supply in certain areas of the world, including southern France, where coinfection with HIV is a problem.

In patients with HIV/*Leishmania* coinfections, there is a high mortality rate due to concurrent illness, complications, and drug toxicity. Pentavalent antimonials and amphotericin B are more toxic to HIV patients, who require close monitoring for pancreatitis, cardiotoxicity, and nephrotoxicity, and these drugs should be avoided if better drugs, such as liposomal formulations of amphotericin B, are available.

Strongyloides stercoralis

In the life cycle of the intestinal nematode *Strongyloides stercoralis*, the larvae migrate through the heart and lungs, pass up the trachea, are swallowed, and finally grow to maturity in the gastrointestinal tract (Tables 30.3, 30.4, and 30.16; Fig. 30.18) (68–77). One significant feature of the *Strongyloides* life cycle is an internal autoinfection capability. Noninfective rhabditiform larvae, which normally pass out in the stool, may transform to infective filariform larvae while still within the gastrointestinal tract or perhaps on the perianal surface. These larvae can then penetrate the bowel wall or skin and reinitiate the life cycle. This cycle can maintain itself at a very low level over a period of many years in an individual without causing symptoms. The only unusual finding in the patient may be an unexplained eosinophilia. Patients who contracted the original infection many years earlier can become severely ill when they become immunosuppressed (Table 30.4). In the compromised patient, the number of larvae and adult worms may increase rapidly, leading to the hyperinfection syndrome and disseminated strongyloidiasis.

Patients in whom systemic *S. stercoralis* infections have been found include those with various leukemias and lymphomas, chronic infections such as leprosy and tuberculosis, and miscellaneous conditions such as kidney disease, organ transplantation, asthma, systemic lupus erythematosus, and various fungal infections. Other conditions include malnutrition, alcoholism, chronic renal failure, and achlorhydria. When migrating *Strongyloides* larvae begin to increase in numbers in the compromised host, the patient may present with abdominal complaints and there may be repeated episodes of unexplained bacteremia or meningitis with enteric bacteria. This occurs when the larvae penetrate the bowel and reinitiate the cycle. In doing so, they carry members of the bowel flora with them. The other consistent finding in these patients is an unexplained eosinophilia, which may range from 10 to 50%. Like *G. lamblia*, *S. stercoralis* resides in the duodenum, and recovery of the larvae in the stool is difficult in patients with low worm burdens. For this reason, additional techniques like the Entero-Test string capsule and the duodenal aspirate may be used. Other techniques for concentrating and recovering *Strongyloides* larvae include the Harada-Mori and petri dish culture techniques, as well as the Baermann apparatus.

TABLE 30.16 *Strongyloides stercoralis* infections in immunocompromised patients[a]

Underlying condition	Disease manifestation	Comments
AIDS and non-Hodgkin's lymphoma	Disseminated disease	After chemotherapy, patient complained of retrosternal pain, dysphagia, dry cough, and upper abdominal discomfort; eosinophilia was 41%; recurrence of lymphoma ruled out; treatment for disseminated atypical mycobacterial infection; biopsy exam of duodenal fluid revealed worms and larvae; albendazole therapy successful
Idiopathic pulmonary fibrosis therapy	Disseminated disease	64-year-old man with pulmonary fibrosis was admitted for shortness of breath and cough; immunosuppressed due to steroids; repeated sputum and stool positive for *S. stercoralis;* died suddenly from severe cardiorespiratory failure while on mebendazole therapy
Nephrotic syndrome therapy	Disseminated disease	Patient with nephrotic syndrome developed disseminated strongyloidiasis after steroid therapy; renal biopsy, time course, resolution of nephrotic syndrome after thiabendazole suggested causal relationship between chronic strongyloidiasis and nephrotic syndrome; early diagnosis important to good clinical outcome
Nephrotic syndrome therapy	Glomerulonephritis	Glomerulonephritis documented
Nephrotic syndrome therapy	Disseminated disease	Problems after administration of steroids
Chronic renal failure	Intestinal disease	Vomiting, diarrhea predominant symptoms; albendazole effective
Immunosuppressive drugs for kidney transplantation	Disseminated disease	Development of strongyloidiasis in renal transplant recipients following cyclosporin A has not been described; *Strongyloides* hyperinfection occurred immediately after cyclosporin A was discontinued; possible transmission by kidney transplant
Adult HTLV-1 infection	Intestinal or disseminated disease	Onset usually follows long period of viral latency; strongyloidiasis is considered a cofactor of leukemogenesis; hypereosinophilia is also seen, could be associated with parasites or leukemic process; cofactors might be important in HTLV-1-associated leukemogenesis; hypereosinophilia affects prognosis
Adult HTLV-1 infection	Intestinal disease	Prevalence of both HTLV-1 and *Strongyloides* increased with age, especially over 50 yr; prevalence of *S. stercoralis* infection significantly higher in HTLV-1 carriers; IgE level low in HTLV-1 carriers and significantly lower in HTLV-1 carriers with *Strongyloides* infection
Adult HTLV-1 infection	Disseminated disease	Strongyloidiasis progresses to hyperinfection or disseminated disease in carriers of HTLV-1; larvae detected in stool; meningitis responded to therapy; larvae still present after thiabendazole; coinfection with *S. stercoralis* is a predisposing factor to meningitis; association may warrant preventive antiparasitic treatment in patients infected with HTLV-1
Adult HTLV-1 infection (Peru)	Disseminated disease including meningitis	*Strongyloides* hyperinfection among Peruvian patients is highly associated with HTLV-1 infection
HTLV-1 infection and ATLL	Intestinal or disseminated disease	ATLL represents ~50% of T-cell lymphomas in Martinique; *Strongyloides* infection during ATLL is linked with high response rate to chemotherapy and prolonged survival; p53 overexpression seen in ~50% of cases of aggressive ATLL from Martinique and is associated with resistance to chemotherapy and short-term survival
HTLV-1 infection	Intestinal disease	*S. stercoralis* hyperinfection syndrome and therapeutic failure in apparently healthy patients with nondisseminated strongyloidiasis may be markers of HTLV-1 infection; HTLV-1 coinfection may also impact clinical course of scabies and HIV disease
Advanced small noncleaved cell lymphoma	Disseminated disease	*S. stercoralis* infection in child mimics relapse (the child was on intensive multiagent chemotherapy for lymphoma, and the treatment was successful in this case); *S. stercoralis* infection in adults being treated for malignant lymphoma is frequently fatal
SLE therapy (active disease)	Disseminated disease	Death due to massive pulmonary hemorrhage induced by filariform infection; diagnosed from bronchoalveolar lavage fluid 2 days before death; importance of checking patients for possible parasitic infections prior to starting therapy, even in areas without endemic infection
Cancer chemotherapy (Mexico)	Intestinal disease	In epidemiologic settings with high prevalence of intestinal parasitic infections, stool exams should be performed and antiparasitic therapy provided before initiating chemotherapy
Ribavirin plus interferon therapy for chronic HCV infection	Disseminated disease	Ribavirin may have immunomodulatory and immunosuppressive action; severe strongyloidiasis reported after use of ribavirin and interferon therapy for HCV infection
Steroids	Intestinal disease	In patients on corticosteroids, vomiting and diarrhea are key symptoms; duodenal mucosa varied from being normal to having severe ulceration; symptoms may be attributed to underlying disease

(continued)

TABLE 30.16 *(continued)*

Underlying condition	Disease manifestation	Comments
Steroids for chronic idiopathic thrombocytopenia	Disseminated disease	Diagnosis difficult due to lack of diarrhea and eosinophilia; negative multiple stool specimens; upper endoscopy revealed larvae, as did sputum specimens; death in spite of thiabendazole therapy
Single-dose steroids prior to radiosurgery	Disseminated disease	Patient received single dose of dexamethasone before stereotactic radiosurgery; fatal disseminated case of strongyloidiasis
Steroids	Intestinal disease (mimicking ulcerative colitis), possibly leading to disseminated disease	*Strongyloides* infection mimics ulcerative colitis over long period; accurate diagnosis is essential
Immunosuppressive drugs	Intestinal disease	Patient with chronic *S. stercoralis* infection initially presented with generalized prurigo nodularis and lichen simplex chronicus (skin nodules, thickened skin)
Diabetes mellitus and malnutrition	Disseminated disease	Repeated vomiting and upper gastrointestinal bleeding; unexplained leukocytosis; pulmonary infiltrates, gastric aspirate leukocytosis, presence of adult worms, eggs, and filariform and rhabditiform larvae in stool specimens; patients died while undergoing treatment with mebendazole
Diabetes mellitus and eosinophilic pleural effusion	Disseminated disease	Fever, exudative type of eosinophilic pleural effusion; bronchial washings contained *S. stercoralis* larvae; patient history extremely important
Unknown	Intestinal disease	Patient admitted for progressive weight loss, abdominal bloating; gastroscopic exam revealed mucosal prepyloric elevations in gastric mucosa; gastric strongyloidiasis confirmed by presence of adult worms, as well as ova and rhabditiform larvae; histologically confirmed case of gastric disease
None (immunocompetent patient)	Eosinophilic pleural effusion	Helminth infections should be considered in cases of eosinophilic pleural effusion
None (immunocompetent patient)	Colitis	Diarrhea, weight loss, microcytic anemia; colonoscopy revealed pancolitis and intense eosinophilic infiltrates; biopsy revealed *S. stercoralis* larvae
None (immunocompetent patient)	Colitis	Poor sensitivity of stool exams, careful search needs to be made in all patients before diagnosis of idiopathic eosinophilic colitis; steroid therapy may have fatal outcome

[a] Adapted from references 68–77. ATLL, adult T-cell leukemia/lymphoma; HCV, hepatitis C virus; SLE, systemic lupus erythematosus.

In individuals with preserved immune function, direct development of *S. stercoralis* is favored, whereas in individuals with lesser immune function, indirect development is relatively more common. These results may explain the notable absence of disseminated strongyloidiasis in patients with advanced HIV disease. Because disseminated infection requires the direct development of infective larvae in the gut, the observed tendency toward indirect development in individuals immunosuppressed by advancing HIV disease is not consistent with the promotion of disseminated infection. However, the two conditions that have been most frequently recognized as predisposing factors for the development of the hyperinfection syndrome are corticosteroid use and human T-lymphotropic virus type 1 (HTLV-1) infection. Reviews of disseminated disease in organ transplant patients, asthmatics, those with chronic lung disease, and those with autoimmune disease indicate that corticosteroid therapy has been a common denominator in the development of severe infection. There also seems to be an association between HIV infection/alcoholism and *S. stercoralis* infection.

The typical rhabditiform larvae of *S. stercoralis* are characterized by a short buccal capsule or mouth opening and the presence of a genital primordial packet of cells. In contrast, the rhabditiform larvae of hookworm have a much longer buccal capsule and essentially no genital primordial cells present.

Whenever one is working with material from a patient suspected of having strongyloidiasis, one should use extreme care because of possible filariform larvae in the specimen. Gloves must be worn to prevent skin penetration by these larval forms.

As we prolong life for more individuals, the medical community must become aware of some of the parasitic

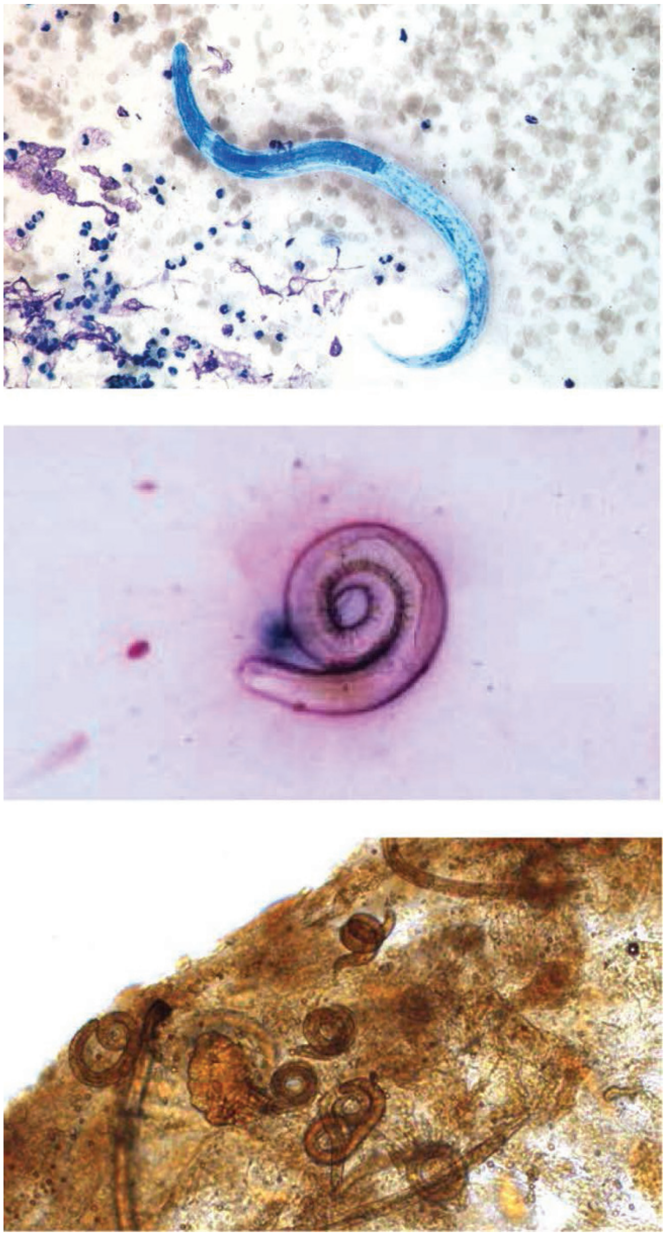


Figure 30.18 *Strongyloides stercoralis*. (Top) Rhabditiform larvae seen in bronchoalveolar lavage fluid specimen, Giemsa stain (larvae can also be seen in sputum in heavy infections or in the hyperinfection syndrome). (Middle) Larva from sputum, Gram stain. (Bottom) Tracheal aspirate containing many larvae (courtesy of Marc Roger Couturier, ARUP Laboratories, Department of Pathology, University of Utah).
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infections that may pose a threat to the life of the compromised patient. Awareness is the first step in preventing more serious disease. Obtaining a migration history is important with each patient; migrant patients may have acute illness or chronic conditions related to exposure in their country of origin. Early detection and treatment for tuberculosis, hepatitis B virus, *Strongyloides*, and schistosomiasis may improve outcomes. Expansion of refugee screening programs to include all migrants should always be considered.

Plasmodium spp.

According to the latest estimates, released in December 2013, there were about 207 million cases of malaria in 2012 (135 million to 287 million) and an estimated 627,000 deaths (range of 473,000 to 789,000). Malaria mortality rates have fallen by 45% globally since 2000 and by 49% in the World Health Organization African Region. Most deaths occur among children living in Africa, where a child dies every minute from malaria.

There are many neglected nonenteric protozoa able to cause serious morbidity and mortality in humans, particularly in the developing world (Tables 30.3, 30.4, and 30.17; Fig. 30.19) (78–83). These diseases are often more severe in the presence of HIV. While information regarding neglected tropical diseases caused by *Plasmodium* is abundant, these protozoa are often not a first consideration in Western countries where they are not endemic. Those at risk include semi-immune HIV-infected women, people with HIV/AIDS, and international travelers from areas of nonendemicity because they lack immunity (immunologically naive).

Coinfection with malaria and HIV in pregnant women is common in sub-Saharan Africa and causes serious sequelae for both mother and newborn child. The increased prevalence and intensity of parasitemia (placental and peripheral infection and parasite density) in HIV-infected women are well known. Similarly, malaria infection seems to be associated with higher viral loads. Women with malaria infection of the placenta also have a higher risk of passing HIV infection to their newborns.

By increasing the malaria parasite biomass in sub-Saharan Africa, the HIV-1 epidemic may also increase the emergence of antimalarial drug resistance, potentially affecting the health of the whole population in countries where both HIV-1 and malaria are endemic. HIV infection is also associated with an increased frequency of clinical malaria and parasitemia. This association tends to become more pronounced with advancing immunosuppression. However, the prevalence of malaria and HIV and the extent of geographic overlap vary widely within each region, even in countries with a high prevalence of both infections. Transmission of malaria and HIV can also result from inadequate blood transfusion practices and, for HIV, the use of unsafe injections. It has also been documented that HIV-infected persons with a low CD4 cell count and anemia have an increased risk of antimalarial treatment failure.

TABLE 30.17 Characteristics of blood parasite infections in immunocompromised patients

Organism	Comments
Plasmodium spp.	Coinfection with malaria and HIV in pregnant women is common in sub-Saharan Africa and causes serious sequelae for both mother and newborn child; women with malaria infection of the placenta also have a higher risk of passing HIV infection to their newborns; HIV infection is also associated with an increased frequency of clinical malaria and parasitemia; HIV-infected persons with a low CD4 cell count and anemia have an increased risk of antimalarial treatment failure
Babesia spp.	Severe disease has been described in HIV-infected patients, even those with intact spleens; immunosuppressed patients with *Babesia* infection may present with systemic complications and may require prolonged courses of antimicrobial therapy to contain and ultimately eradicate infection; patients who do not respond to a course of standard therapy are likely to have medical conditions that severely impair immune function; complications in these patients include anemia severe enough to require a blood transfusion or exchange transfusion, the presence of pulmonary edema or acute respiratory distress syndrome, and/or impairment of renal function severe enough to require dialysis.
Trypanosoma cruzi, American trypanosomiasis	Chagas' disease caused by *Trypanosoma cruzi* is a well-recognized opportunistic infection associated with AIDS; *T. cruzi* infection has been classified as an AIDS-defining opportunistic parasitosis by the Centers for Diseases Control and Prevention since 2004; the most common symptoms of Chagas' disease reactivation are subcutaneous nodules (chagoma), panniculitis, myocarditis with signs of heart failure, fever, meningitis, encephalitis, and stroke, but other symptoms, such as fatigue, anorexia, and diarrhea, are also seen; immunosuppressed patients with chronic infection can develop reactivations, which can be very severe and are associated with high mortality

When the diagnosis of malaria is considered, only a single blood specimen may be submitted to the laboratory for examination; however, single films or specimens cannot be relied on to exclude the diagnosis, especially when partial prophylactic medication or therapy is used. Partial use of antimalarial agents may be responsible for reducing the numbers of organisms in the peripheral blood, thus leading to a blood smear that contains few organisms, which then reflects a low parasitemia when in fact serious disease is present. Patients with a relapse case or an early primary case may also have few organisms in the blood smear.

It is recommended that both thick and thin blood films be prepared on admission of the patient, and at least 200 to 300 oil immersion fields should be examined on both films before a negative report is issued. Since one set of negative films will not rule out malaria, additional blood specimens should be examined over a 36-h time frame. Although Giemsa stain is recommended for all parasitic blood work, the organisms can also be seen if other blood stains, such as Wright's stain, are used. Blood collected with the use of EDTA anticoagulant is acceptable; however, if the blood remains in the tube for any length of time, true stippling may not be visible within the infected RBCs (*Plasmodium vivax*, as an example). Also, when using anticoagulants, it is important to remember that the proper ratio between blood and anticoagulant is necessary for good organism morphology. Heparin can also be used, but EDTA is preferred.

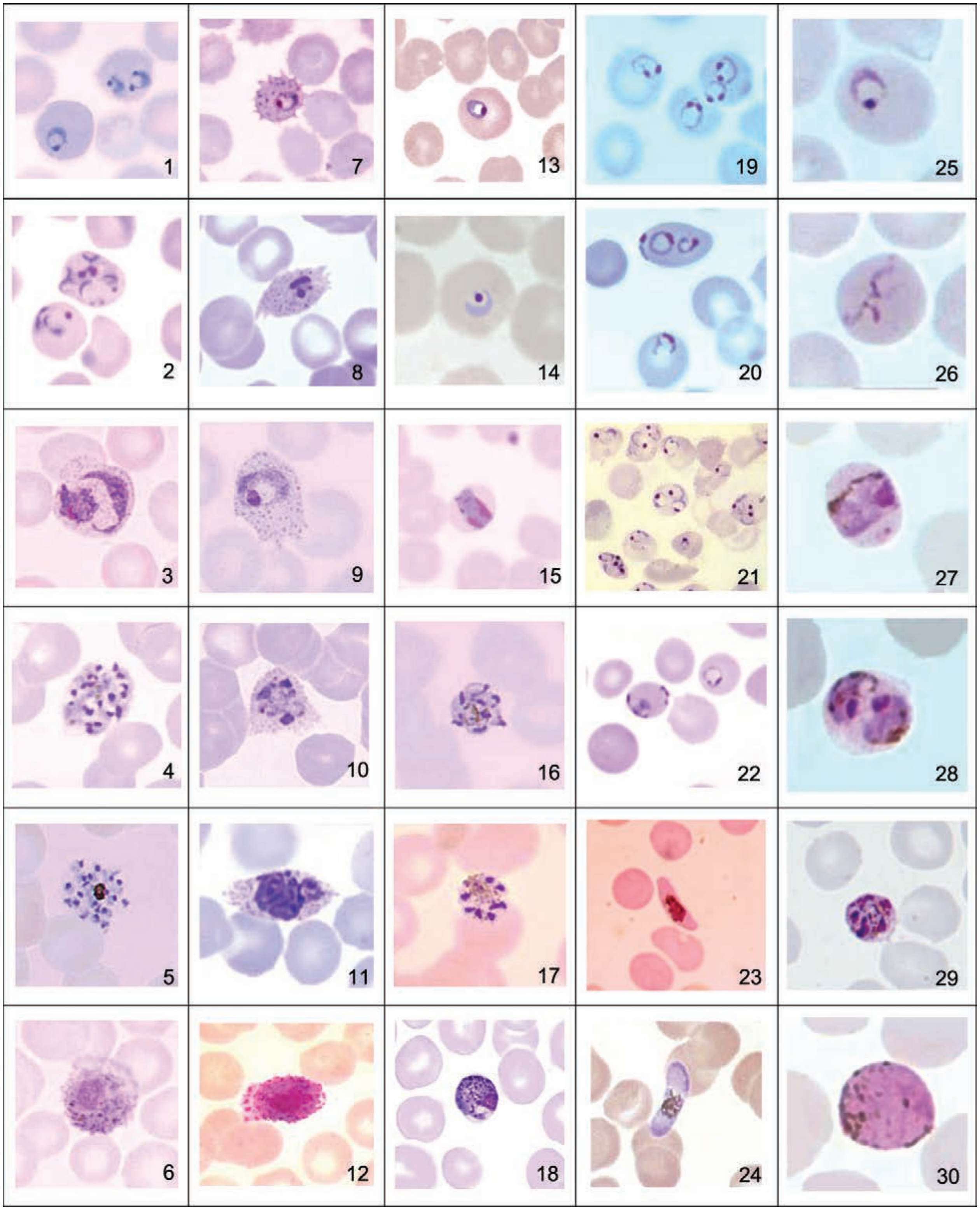
Sensitivity of the rapid malaria tests remains a problem, particularly for nonimmune populations. Parasite densities of >100 parasites/μl (0.002% parasitemia) should be detected and are reasonable targets to expect from dipsticks for *Plasmodium falciparum* diagnosis. However, this level of sensitivity is at the lower end of the capability of most devices using capture methods for HRP-2 or pLDH. Currently, the BinaxNOW Malaria Test is FDA approved (Alere, Inc., Waltham, MA).

Malaria is one of the few parasitic infections considered to be immediately life-threatening, and a patient with the diagnosis of P. falciparum *or* P. knowlesi *malaria should be considered a medical emergency because the disease can be rapidly fatal. Any laboratory providing the expertise to identify malarial parasites should do so on a 24-h basis, 7 days per week.*

Babesia spp.

Intraerythrocytic protozoa of the genus *Babesia* parasitize numerous vertebrates, predominantly in tropical and subtropical regions. Human disease has mostly been reported from temperate zones. Severe disease has been described in HIV-infected patients with one of the temperate species causing malaria-like disease in humans, raising the possibility that tropical species present a risk for patients with AIDS. Severe, persistent, and/or recrudescent *Babesia microti* parasitemia has occurred in immunosuppressed HIV-infected patients with intact spleens (Tables 30.3, 30.4, and 30.17; Fig. 30.20) (84–86). Babesiosis should be considered in the differential diagnosis of HIV-infected patients with fevers and/or anemia in areas where the infection is endemic. HIV-infected patients who are severely immunosuppressed, even those without a history of splenectomy, may present with severe manifestations of babesiosis and develop a chronic infection, which may require therapy to prevent relapse of disease. The inability of the innate immune system to clear parasites results in high levels of circulating parasitized RBCs.

The mechanism of parasitic persistence has not been totally delineated. A number of studies have implicated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gamma interferon (IFN-γ) as one of the critical cytokines for control of the early stages of infection. Natural killer cells are a major source of IFN-γ; however, in HIV-infected patients less IFN-γ is secreted. Additionally, T cells have been shown to play a critical role in the resolution of babesiosis.

In one study of immunosuppressed patients with babesiosis, most case patients experienced B-cell lymphoma and were asplenic or had received rituximab before babesial illness. There may be a direct association between certain primary or acquired immunodeficiency syndromes and the failure to contain acute babesial illness. These immunosuppressed patients with *Babesia* infection may present with systemic complications and may require prolonged courses of antimicrobial therapy to contain and ultimately eradicate infection. Patients who do not respond to a course of standard therapy are likely to have medical conditions that severely impair immune function.

Diagnosis of *B. microti* infection is normally based on the presence of symptoms consistent with babesiosis and the recognition of organisms by microscopic examination of stained thick and thin blood films. Symptoms include fever, chills, sweats, fatigue, headache, and myalgia. Complications in these patients include anemia severe enough to require a blood transfusion or exchange transfusion, the presence of pulmonary edema or acute respiratory distress syndrome, and/or impairment of renal function severe enough to require dialysis.

American Trypanosomiasis

One of the most prevalent parasitic infections that have spread from Latin America to areas of nonendemicity such as the United States, Canada, and Europe is Chagas' disease, or American trypanosomiasis. Its etiological agent, *Trypanosoma cruzi*, has not traditionally been considered an opportunistic agent, but in recent decades there have been many reports of reactivation in immunocompromised patients. Chagas' disease caused by *T. cruzi* is a well-recognized opportunistic infection associated with AIDS. *T. cruzi* infection has been classified as an AIDS-defining opportunistic parasitosis by the CDC since 2004. To date, the cases reported largely represent reactivation of chronic infection, which is most commonly manifested by meningoencephalitis (Tables 30.3, 30.4, 30.17; Fig. 30.21) (87–92). Reactivation is defined as an increase in parasitemia that can be detected by diagnostic methods, even in patients who are asymptomatic. The appearance of symptoms represents a worsening of the underlying disease. The most common symptoms of Chagas' disease reactivation are subcutaneous nodules (chagoma), panniculitis, myocarditis with signs of heart failure, fever, meningitis, encephalitis, and stroke, but other symptoms, such as fatigue, anorexia, and diarrhea, are also seen.

T. cruzi infection occurs primarily in rural regions, and HIV infection occurs primarily in urban regions. Clinical reactivation generally occurs in persons with CD4$^+$ T-cell counts <200 cells/mm^3 and most commonly involves the CNS. Lesions are often multiple, with preferential involvement of the white matter. Hemorrhagic foci can produce mass effects simulating brain tumors. Histologically, brain lesions exhibit necrosis, hemorrhage, and inflammatory infiltrates. Amastigote forms of the parasite are abundant in glial cells and macrophages. Myocarditis is a common autopsy finding in persons who have died of meningoen-

Figure 30.19 Morphology of malaria parasites. Column 1 (reading down), *Plasmodium vivax* (note enlarged infected RBCs): (1) early trophozoite (ring form) (note one RBC contains two rings—not that uncommon); (2) older ring, note ameboid nature of rings; (3) late trophozoite with Schüffner's dots (note enlarged RBC); (4) developing schizont; (5) mature schizont with 18 merozoites and clumped pigment; (6) microgametocyte with dispersed chromatin. Column 2, *Plasmodium ovale* (note enlarged infected RBCs): (7) early trophozoite (ring form) with Schüffner's dots (RBC has fimbriated edges); (8) early trophozoite (note enlarged RBC, Schüffner's dots, and RBC oval in shape); (9) late trophozoite in RBC with fimbriated edges; (10) developing schizont with irregular-shaped RBC; (11) mature schizont with eight merozoites arranged irregularly; (12) microgametocyte with dispersed chromatin. Column 3, *Plasmodium malariae* (note normal or smaller than normal infected RBCs): (13) early trophozoite (ring form); (14) early trophozoite with thick cytoplasm; (15) late trophozoite (band form); (16) developing schizont; (17) mature schizont with nine merozoites arranged in a rosette; (18) macrogametocyte with compact chromatin. Column 4, *Plasmodium falciparum*: (19) early trophozoites (the rings are in the headphone configuration with double chromatin dots); (20) early trophozoite (accolé or appliqué form); (21) early trophozoites (note the multiple rings per cell); (22) late trophozoite with larger ring (accolé or appliqué form); (23) crescent-shaped gametocyte; (24) crescent-shaped gametocyte. Column 5, *Plasmodium knowlesi* (with the exception of image 29, these were photographed at a higher magnification; note normal or smaller than normal infected RBCs; courtesy of the CDC Public Health Image Library): (25) early trophozoite (ring form); (26) early trophozoite with slim band form; (27) late trophozoite (band form); (28) developing schizont; (29) mature schizont with merozoites arranged in a rosette; (30) microgametocyte with dispersed chromatin. Note: Without the appliqué form, Schüffner's dots, multiple rings per cell, and other developing stages, differentiation among the species can be very difficult. It is obvious that the early rings of all four species can mimic one another very easily. Remember: One set of negative blood films cannot rule out a malaria infection. (Figure courtesy of Garcia LS, *Clin Lab Med* **30**:93–128, 2010.) doi:10.1128/9781555819002.ch30.f19

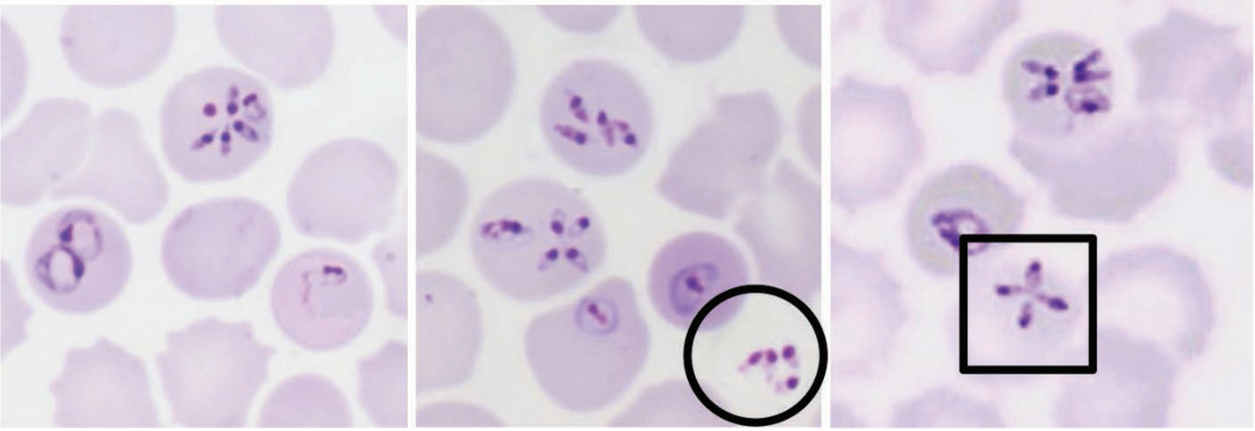

Figure 30.20 Three examples of blood films containing the ringlike forms of *Babesia* spp. Various ring forms, multiple rings per cell; some rings present outside of the red blood cells (circle); note the typical image of the "Maltese cross" configuration of the four rings (square). Note: it is rare to see *Plasmodium* rings outside of the RBC.
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cephalitis. Such myocarditis is often clinically silent. When present, clinical manifestations include arrhythmias and congestive heart failure. Immunosuppressed patients with chronic infection can develop reactivations, which can be very severe and are associated with high mortality.

The higher levels of parasitemia observed in HIV-infected individuals with symptoms of Chagas' disease are useful in diagnosis. Direct microscopic examination of blood and CSF may reveal *T. cruzi* trypomastigotes. This disease confirmation may precede clinical symptoms or it may be discovered with the onset of symptoms.

If fever and other systemic signs are present, direct examination of the buffy coat may also reveal motile trypanosomes. Blood culture and xenodiagnosis are of considerable use; the latter is generally unavailable outside of regions of endemicity.

Crusted Scabies

Scabies is a highly contagious infestation of the itch mite, *Sarcoptes scabiei*. Symptoms are related to pruritic dermatitis, and apparently transmission can occur through person-to-person direct skin contact or via fomites (Tables 30.3 and 30.4 and Fig. 30.22) (93–95). Outbreaks of scabies in hospitals or institutions, particularly for the elderly or developmentally disabled, have been documented worldwide. These outbreaks are particularly severe when associated with crusted scabies; such patients may be infected with thousands of mites on the skin. This infection has been linked with cellular immune deficiencies, including AIDS. Other potential problems include a decreased awareness of itching and the use of topical corticosteroids to reduce itching. Because of the number of mites present, these patients are extremely contagious, from sloughing skin, direct contact, and environmental contamination. Another potential problem is the fact that the symptoms mimic those of many other dermatologic conditions. Burrows may not be evident, and hyperkeratotic, crusted, scaling, fissured plaques are present over the scalp, face, and back, with associated gross nail thickening. The number of documented reports, particularly in AIDS patients, suggests that crusted scabies may become more common in the future.

Crusted scabies should be considered in the differential diagnosis of a generalized cutaneous eruption in a HTLV-1-positive patient. Patients with crusted scabies from an HTLV-1-endemic population should be tested for a possible HTLV-1 infection. Infection with HTLV-1 is an important cofactor related to crusted scabies in Peru. Testing for HTLV-1 in all crusted scabies cases is highly recommended, especially when no other risk factors are apparent. These patients may be at increased risk of progressing to adult T-cell leukemia/lymphoma. Definitive parasitic diagnosis can be difficult; difficulties in management have led to renewed interest in both scabies and pediculosis. The diagnosis of scabies should always be considered in patients with advanced malignancies and associated pruritus.

The diagnosis of crusted scabies can easily be missed. Serpiginous tracks were noted on the surface of Sabouraud's dextrose agar used for fungal culture of the skin scrap-

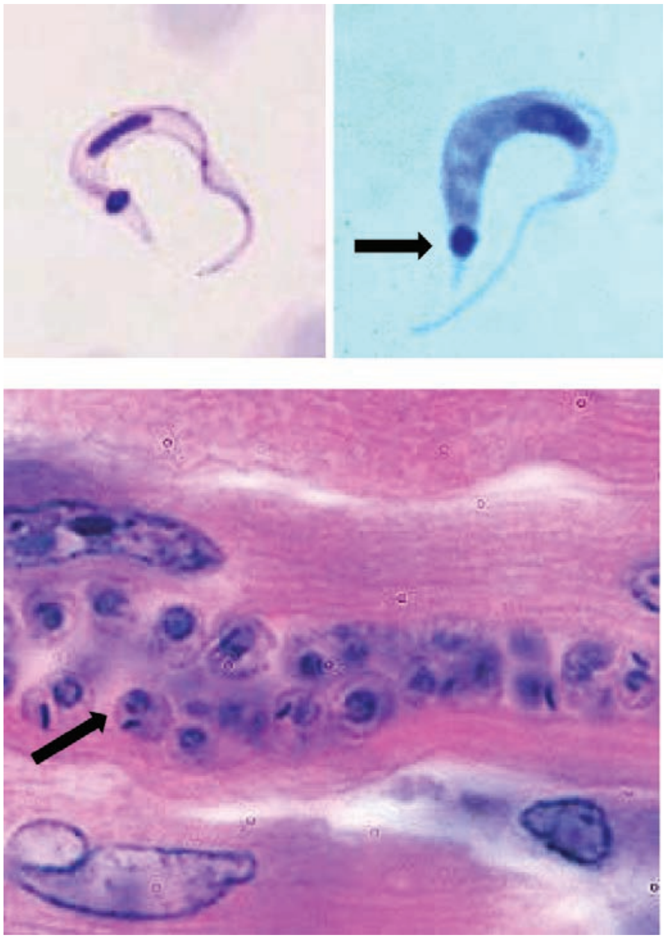

Figure 30.21 (Upper) *Trypanosoma cruzi* trypomastigotes in a peripheral blood smear; note the large kinetoplast (arrow). (Lower) Amastigote in cardiac muscle; note the actual amastigote with nucleus and kinetoplast (arrow).
doi:10.1128/9781555819002.ch30.f21

ings from an elderly long-term-care facility resident. This unusual laboratory manifestation alerted clinical microbiologists to the possible diagnosis of scabies. Although many microbiology laboratories are aware of these unusual findings, personnel can forget to consider scabies in such situations.

Ivermectin is increasingly being used to treat scabies, especially crusted scabies. However, treatment failures, recrudescence, and reinfection can occur, even after administration of multiple doses. Clinical and *in vitro* evidence of ivermectin resistance has been found in several patients with multiple recurrences of crusted scabies who had previously received 30 to more than 50 doses of ivermectin over a 4-year time frame. As predicted, ivermectin resistance in scabies mites can develop after intensive ivermectin use.

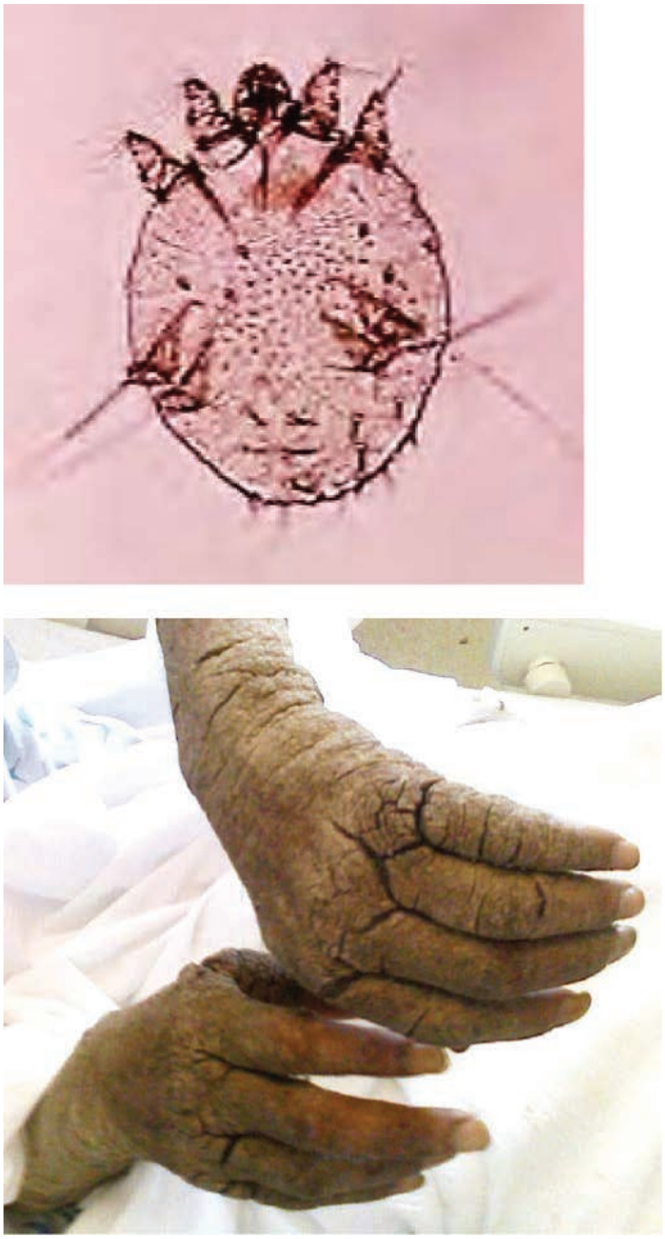

Figure 30.22 *Sarcoptes scabiei* "itch mite." (Upper) Mite from skin scraping preparation (note the four pairs of legs). (Lower) Hands of a homeless individual with AIDS and severe scabies (crusted scabies) (courtesy of the Wikimedia Commons, Adam Cuerden; released into the public domain by author).
doi:10.1128/9781555819002.ch30.f22

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31

Health Care-Associated and Laboratory-Acquired Infections

Health Care-Associated infections

Gastrointestinal infections

Cryptosporidium spp.
Giardia lamblia
Entamoeba histolytica
Microsporidia
Cystoisospora (*Isospora*) *belli*
Hymenolepis nana
Taenia solium

Blood and tissue infections

Plasmodium spp.
Babesia spp.
Trypanosoma brucei gambiense and *T. brucei rhodesiense*
Trypanosoma cruzi
Leishmania donovani
Toxoplasma gondii

Infections with ectoparasites

Pediculus spp. and *Phthirus pubis*
Sarcoptes scabiei
Myiasis

Infections in the pediatric patient

Cryptosporidium spp.
Giardia lamblia
Pediculus humanus capitis
Sarcoptes scabiei

Infections in the compromised patient

Laboratory infections

Intestinal protozoa
Free-living amebae
Plasmodium spp.
Trypanosoma brucei gambiense and *T. brucei rhodesiense*
Trypanosoma cruzi
Leishmania spp.
Toxoplasma gondii
Specimen handling

Health Care-Associated (Nosocomial) Infections

Nosocomial infections are those that are hospital acquired or hospital associated. According to some publications, nosocomial infections are estimated to complicate the course of 5 to 10% of all hospitalized patients in acute-care facilities (1, 2). These infections are a significant cause of morbidity, mortality, and increased hospital costs, largely as a result of the increased length of stay. Over the past few years, escalating medical costs and the introduction of outpatient surgery have resulted in shorter hospital stays and much higher volumes of outpatient surgical procedures. Unfortunately, these changes have tended to mask true surgical infection rates. Also, postoperative surgical-site infections are often not apparent until after the patient's discharge (3). Since 1976, hospital accreditation by the Joint Commission on Accreditation of Healthcare Organizations (now The Joint Commission) requires the use of a nurse epidemiologist or infection control nurse whose primary responsibility is to identify, track, and control infections associated with hospitalization (4).

Most studies of the incidence of health care-associated infections ignore parasitic infections. Based on data from 1,265 intensive care units in 75 countries, it was found that the proportion of parasites in these infections was 0.48% overall and 0.25% in Western Europe (5). Parasites have been observed to be etiological agents of diarrhea in 12 to 17% of health care-associated epidemics and 1% of endemic outbreaks (5), this figure being higher in surgical wards (19.8%) (6, 7); protozoa such as *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia lamblia*, and *Blastocystis* spp. were detected most frequently.

Individuals in the institution who fall within the surveillance parameters of the nurse epidemiologist include both patients and employees. Although we usually consider patients to be more prone to infections, some microorganisms can also infect healthy adults (8).

Definition: A health care-associated infection is defined as one diagnosed during or after hospitalization that was neither present nor incubating at the time of admission (1, 2).

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One of the problems with this definition is the difficulty involved in accurately differentiating community-acquired from hospital-acquired infections, particularly when employees are involved. Depending on the individual's immune status, incubation periods may also vary.

Although any organism can infect a susceptible host, the organisms most often considered to cause possible nosocomial infections, particularly in the immunologically compromised, include the following:

Fungi: *Aspergillus* spp., *Candida* spp., *Mucor* spp., *Cryptococcus neoformans*, *Nocardia asteroides*

Algae: *Prototheca* spp.

Bacteria: *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Legionella* spp.

Viruses: Cytomegalovirus, varicella-zoster virus, HIV, rubella virus, rotavirus, enterovirus

Parasites: *Giardia lamblia*, *Entamoeba histolytica*, free-living amebae, *Toxoplasma gondii*, *Cryptosporidium* spp., the microsporidia, *Strongyloides stercoralis*, *Trypanosoma* spp., *Plasmodium* spp., *Babesia* spp., *Hymenolepis nana*, *Taenia solium*

Ectoparasites: *Pediculus* spp., *Sarcoptes scabiei*, dipterous fly larvae (myiasis)

Although most health care-acquired infections are bacterial, fungal, or viral in origin, a few can be caused by parasites. The patient may actually have acquired the infection (*T. gondii*, *S. stercoralis*) many years before hospitalization. These latent infections can often be reactivated when the patient becomes debilitated for some reason, particularly when the immune system is involved.

Other areas within an institution must be considered, including interaction between food service personnel and patients and employees. Public health requirements for checking food handlers have varied over the years, but as a result of cost containment measures, the general trend is to refrain from special laboratory work or routine screening procedures for employees. Parasite transmission possibilities are presented in Table 31.1. Not every organism is listed; however, those that represent actual possibilities for transmission are emphasized.

Sometimes patients are placed in isolation settings (enteric precautions), which may not be appropriate or necessary for the organism or infection involved. A good example is a patient with amebic dysentery. If the patient has diarrhea or dysentery, the organism stage being passed in the stool is the trophozoite. The trophozoite stage is noninfectious for others. The cyst form, which is passed in more normal, formed stools, is the infective stage for humans. The trophozoites do not form cysts after the stool is passed outside of the body. Therefore, this patient with amebic dysentery does not pose an infection threat to others.

An example in which the need for enteric precautions would be recommended is a patient with diarrhea caused by *Cryptosporidium*. The oocysts are immediately infective on passage, and often large numbers of organisms are passed in the stool, which may vary from formed to watery. There are several reports of suspected transmission of *Cryptosporidium* from patients to health care workers (9–13).

Infection with blood and tissue parasites can occur by accidental needlestick, contact with blood (open wound, mucous membranes, eye), transfusion, or organ transplantation (14). Such transmission of babesiosis has also been documented (15).

Gastrointestinal Infections

Nosocomial gastroenteritis can be defined as an acute infectious gastrointestinal illness acquired by a hospitalized patient, specifically unexplained diarrhea for 2 days, or the onset of infectious diarrhea somewhere in the hospital or medical center setting. In general, requests for ova and parasite identification are not indicated for patients with nosocomial diarrhea (16). Patients at highest risk for gastrointestinal infections include neonates, the elderly, patients with achlorhydria, and those who are immunosuppressed (16). Risk factors can be categorized as those within the host and those outside the host. Risk factors within the host include impaired immunity, reduced gastric acidity, and alterations in the normal intestinal flora or intestinal motility. Risk factors outside the patient include those that alter host resistance or increase the possibility of colonization. The transmission of organisms is generally via the fecal-oral route; some of the organisms can survive for long periods outside the body. Parasites that have been implicated in health care-associated infections include *Cryptosporidium* spp., *E. histolytica*, and *G. lamblia*. Although little is known about transmission of microsporidia, potentially infectious spores can be found in the hospital environment, particularly in stools from severely immunocompromised patients (e.g., those with AIDS). The presence of infective spores in human clinical specimens suggests that precautions when handling body fluids and personal hygiene measures such as hand washing may be important in preventing primary infections in the health care setting. However, comprehensive guidelines for disease prevention will require more definitive information regarding sources of infection and modes of transmission (17).

Cryptosporidium spp.

Although cryptosporidiosis is a well-recognized occupational hazard for persons exposed to naturally infected calves and other animals, cases of cryptosporidiosis have also been reported among persons exposed to experimentally infected animals (18). Five veterinary students who

TABLE 31.1 Possible parasite transmission in a health care setting (nonlaboratory)

Organism[a]	Mode of transmission	Comments
Protozoa (intestinal)		
Entamoeba histolytica[b] (C)	Accidental ingestion of infective cysts, trophozoites, oocysts, or spores in food or water contaminated with fecal material (fecal-oral transmission)	These organisms could be important in a food handler situation. Treatment of carriers and excellent hygiene measures would prevent or eliminate any problems. *Cryptosporidium* is an example where "enteric precautions" would be appropriate (severe diarrhea with stool containing large numbers of infective oocysts).
Entamoeba dispar (C)		
Entamoeba coli (C)		
Entamoeba hartmanni (C)		
Endolimax nana (C)		
Iodamoeba bütschlii (C)		
Blastocystis spp.[b] (C)		
Giardia lamblia[b] (C)		
Dientamoeba fragilis[b] (T)		
Cryptosporidium spp.[b] (O)		
Cyclospora cayetanensis[b] (O)		
Cystoisospora belli[b] (O)		
Microsporidia[b] (S)		
Helminths (intestinal)		
Enterobius vermicularis (E)	Inhalation or ingestion of infective eggs	Very common in children, asymptomatic
Strongyloides stercoralis (IL)	Skin penetration of infective larvae from stool material	Possible, particularly in a compromised patient
Hymenolepis nana (E)	Ingestion of infective eggs (fecal-oral)	Could lead to infection with the adult worm in humans
Taenia solium (E)	Ingestion of infective eggs (fecal-oral)	Could cause cysticercosis
Blood and tissue protozoa		
Leishmania spp. (cutaneous)	Direct contact or inoculation of infectious material from patient's lesion could lead to infection; blood transfusion; organ transplantation	A rare possibility, but it has been documented (accidental person-to-person transmission)
Trypanosoma spp.	Blood transfusion, organ transplantation	Significant problem in areas of endemicity
Toxoplasma gondii	Blood transfusion, organ transplantation	
Ectoparasites		
Pediculus spp.	Transmission of lice and mites is usually seen in groups with very close contact (institutionalized settings).	Confinement in close quarters often lends itself to a transmission problem with lice and mites.
Sarcoptes scabiei		
Dipterous fly larvae	Myiasis could occur anywhere.	Protection of wounds from flies would solve the potential problem.

[a] C, cyst; T, trophozoite (may be transmitted inside helminth eggs); O, oocyst; E, egg; IL, infective larvae; S, spore.

[b] Potentially pathogenic intestinal protozoa.

had direct (four) or indirect (one) contact with experimentally infected calves became ill 6 to 7 days later and had diarrhea for 1 to 13 days; one student was hospitalized. A researcher developed gastrointestinal symptoms 5 days after a rabbit, which was infected with oocysts through a gastric tube, coughed droplets of inoculum onto his face as he was removing the tube. The researcher's stool, which was first obtained for testing the day after he became ill, was positive for oocysts. A veterinary scientist developed flu-like symptoms 7 days after smelling for gastric odor to check the position of a gastric tube in an infected calf; she was unaware of other exposures to *Cryptosporidium*. She developed gastrointestinal symptoms 10 days after this exposure, and oocysts were found in a stool specimen about a week later.

Cryptosporidium can also be transmitted within the hospital setting. At least nine cases of occupational transmission of *Cryptosporidium* from human patients to health care workers have been reported (18). The infected staff members were symptomatic and had oocysts identified from stool examinations. These individuals included a nurse caring for an infected bone marrow transplant recipient, a nurse doing night duty on a ward where an infected 13-month-old boy was a patient, a nurse caring for infected patients before and after renal transplantation, and five nurses caring for an infected patient with AIDS. Patient-to-patient transmission of *Cryptosporidium* in hospitals has also been reported.

Cryptosporidiosis has been implicated as one of the more important opportunistic agents seen in patients with AIDS (19–21). One of the most interesting cases was such an outbreak in a hospital in Copenhagen. As many as 18 HIV-positive patients in a short time were infected with *Cryptosporidium* spp. Epidemiological investigation showed that the source of the parasite was a patient with cryptosporidiosis and a history of psychosis, who, through frequent use of the ice machines, contaminated ice cubes with *Cryptosporidium* oocysts. These oocysts are very resistant to environmental conditions, even on a dry surface. Thus, contamination of ice in a very short period of time resulted in the spread of the pathogen among people with impaired immunity (22).

Unfortunately, at the time of this writing, no totally effective therapy for cryptosporidiosis has been identified, despite testing of over 100 drugs. Consequently, finding this parasite in an immunocompromised host, especially one with AIDS, usually carries a poor prognosis. Also, reports of respiratory tract and biliary tree infections confirm that the developmental stages of this organism are not always confined to the gastrointestinal tract. The more than 4,000 published papers related to cryptosporidiosis that are currently in the literature include information on health care-related transmission, day care center outbreaks, and a number of waterborne outbreaks.

The presence of a thin-walled autoinfective oocyst may explain why a small inoculum can lead to an overwhelming infection in a susceptible host and why immunosuppressed patients may have persistent, life-threatening infections in the absence of documentation of repeated exposure to oocysts. Oocysts undergo sporogony while they are in the host cells and are immediately infective when passed in the stool. In contrast, the oocysts of *Cyclospora cayetanensis* and *Cystoisospora belli* do not sporulate until they are passed from the host and are exposed to oxygen and temperatures below 37°C.

Oocysts recovered in clinical specimens are difficult to see without special stains, such as the modified acid-fast, Kinyoun's, or Giemsa preparations, or the direct fluorescent-antibody, enzyme-linked immunosorbent assay, or immunochromatographic immunoassay methods (23–26). The four sporozoites can be seen within the oocyst wall in some of the organisms, although they are not always visible in freshly passed specimens.

Clinical symptoms include nausea, low-grade fever, abdominal cramps, anorexia, and 5 to 10 watery, frothy bowel movements a day, which may be followed by constipation. Some patients present with diarrhea as described above, and others have relatively few symptoms, particularly later in the course of the infection. In patients with the typical watery diarrhea, the stool specimen contains very little fecal material and is mainly water and mucus flecks. Often, the organisms are entrapped in the mucus, and diagnostic procedures are performed accordingly. Generally a patient with a normal immune system will have a self-limited infection; however, patients who are compromised may have a chronic infection with a wide range of symptoms (asymptomatic to severe).

Occasionally, these patients require fluid replacement, and the diarrhea may persist for more than 2 weeks. This is particularly true in infants, in whom excessive fluid loss may last for more than 3 weeks. Failure to thrive has also been attributed to chronic cryptosporidiosis in infants. Since diarrheal illness is a major cause of morbidity and mortality in young children living in developing countries, it is likely that cryptosporidiosis plays a major role in the overall health status of these children. It has also been suggested that *Cryptosporidium* may be implicated in the respiratory disease that often accompanies diarrheal illness in malnourished children.

The duration and severity of diarrheal illness depend on the immune status of the patient. The most severely immunocompromised patients cannot self-cure, the illness becomes progressively worse with time, and the sequelae may be a major factor leading to death. The length and severity of illness may also depend on the ability to reverse the immunosuppression. In these patients, *Cryptosporidium* infections are not always confined to the gastrointestinal tract; additional symptoms have been associated with extraintestinal infections (respiratory problems, cholecystitis, hepatitis, and pancreatitis). Data for a small series of patients indicated that those with CD4 counts of 180 cells/mm^3 or greater cleared the *Cryptosporidium* infection over a period of 7 days to 1 month. These data are important in predicting the natural progression of the infection and in designing therapeutic trials.

Previously, most human cases have been diagnosed after examination of small- or large-bowel biopsy material, often by both light and electron microscopy. Although examination of flotation material by phase-contrast microscopy has proven to be an excellent procedure for the recovery and identification of *Cryptosporidium* oocysts, many laboratories have neither access to such equipment nor experience with phase-contrast microscopy. Also, since the organism is considered infectious for laboratory personnel, fixed specimens can be processed by using several acid-fast stains, many of which are very satisfactory in demonstrating the organisms in stool material (27).

Respiratory cryptosporidiosis has been found in AIDS patients. Sputum specimens from immunodeficient patients with undiagnosed respiratory illness should be submitted in 10% formalin and examined for *Cryptosporidium* oocysts by the same techniques as those used for stool samples. Infection of the gallbladder and biliary tree should result in oocysts being passed in the stool.

The use of fecal immunoassays has proven to be very helpful in providing more sensitive methods of detecting organisms in stool specimens. Fluorescent-antibody procedures with good specificity and sensitivity have been developed, as have enzyme immunoassay procedures and immunochromatographic cartridges, also with excellent specificity and sensitivity. Some of these reagents, particularly the combination direct fluorescent-antibody product used to identify both *Giardia* cysts and *Cryptosporidium* oocysts, are being widely used in water testing and outbreak situations (23–26).

The infections tend to be self-limiting in patients who have an intact immune system (28). One method of treating patients who are receiving immunosuppressive drugs is to discontinue such a regimen. Other approaches with specific therapeutic drugs such as spiramycin have been tried, but the results to date are somewhat controversial. Data indicate that paromomycin may be a potential therapeutic agent for extended clinical trials. Ongoing studies of immunologic intervention hold promise, but this technique currently is not widely available.

Information on the epidemiology of cryptosporidiosis is well defined, and the potential pathogenicity of *Cryptosporidium* in humans has been widely recognized. The organism is transmitted by oocysts that are fully sporulated and infective at the time they are passed in stool. Oocysts are resistant to some of the disinfectants routinely used in medical care facilities. They are susceptible to ammonia, 10% formalin in saline, freeze-drying, and exposure to temperatures below freezing or above 65°C for 30 min. Commercial bleach in a 50% solution is effective.

Direct person-to-person transmission is likely and may occur through direct or indirect contact with stool material. Direct transmission may occur during sexual practices involving oral-anal contact. Indirect transmission may occur through exposure to positive specimens in a laboratory setting or through exposure to contaminated surfaces, food, or water.

The epidemiologic considerations for cryptosporidiosis emphasize transmission by environmentally resistant oocysts, numerous potential reservoir hosts for zoonotic transmission, documentation of person-to-person transmission in day care centers, transmission within the health care setting, occurrence of asymptomatic infections (infective, carrier state), widespread environmental distribution resulting in the probability of waterborne transmission, and the link between cryptosporidiosis and severe, life-threatening disease in individuals with impaired immune function.

Giardia lamblia

A "debilitating bout" of giardiasis thought to have represented patient-to-staff transmission has been reported. The case involved an orthopedic surgeon caring for two preschool-age patients with giardiasis. In changing the leg cast of one child, the physician noted that the cast was noticeably stained with moist and dry feces. The physician became ill approximately 2 weeks later and had giardiasis confirmed with routine stool examinations. Although he typically washed his hands before and after changing casts, he rarely wore a mask; infection may have been caused by inhalation of infectious cysts in the plaster dust.

G. lamblia is worldwide in distribution and is apparently more prevalent in children than in adults and more common in warm climates than in cool ones. It is the most commonly diagnosed flagellate in the intestinal tract, and it may be the second most commonly diagnosed intestinal protozoan in some areas of the world, with *Blastocystis* being first. Despite disagreement concerning the species names *intestinalis* and *duodenalis*, both continue to be used to describe this organism. Since the majority of health care workers within the United States are used to "*Giardia lamblia*" and continue to report the presence of the organism by using this name, it will continue to be used throughout this text to refer to organisms found in humans as well as other mammals.

The most common location of the organisms is in the crypts within the duodenum. Again, for reasons that are not totally known, cyst formation takes place as the organisms move down through the colon. The trophozoite is usually described as being teardrop shaped from the front, with the posterior end being pointed. When examined from the side, the trophozoite resembles the curved portion of a spoon. The concave portion is the area of the sucking disk. The cysts may be either round or oval, and they contain four nuclei, axonemes, and median bodies. Often, some cysts appear to be shrunk or distorted, and one may see two halos, one around the cyst wall itself and one inside the cyst wall around the shrunken organism. The halo effect around the outside of the cyst is particularly visible on the permanent stained smear.

Although the organisms in the crypts of the duodenal mucosa may reach very high densities, they may not cause any pathologic changes. The organisms feed on the mucus secretions and do not penetrate the mucosa. Although organisms have been seen in biopsy material inside the intestinal mucosa, others have been seen only attached to the epithelium. In symptomatic cases, there may be irritation of the mucosal lining, increased mucus secretion, and dehydration. Other symptoms include epigastric pain, flatulence, and diarrhea with increased fat and mucus in the stool but no blood. Weight loss often accompanies these symptoms. Although there is speculation that the organisms coating the mucosal lining may act to prevent fat absorption, this does not completely explain why the uptake of other substances normally absorbed at other intestinal levels is prevented. Occasionally, the gallbladder is also involved, causing gallbladder colic and jaundice. *G. lamblia* has also been detected in bronchoalveolar lavage fluid.

Although patients with symptomatic giardiasis usually have no underlying abnormality of serum immunoglobulins, patients with immunodeficiency syndromes, particularly common variable hypogammaglobulinemia, have a high incidence of giardiasis. Giardiasis was the most common cause of diarrhea in these patients and was associated with mild to severe villous atrophy. Successful treatment of giardiasis led to symptomatic cure and improvement in mucosal abnormalities, with the exception of nodular lymphoid hyperplasia.

With the advent of AIDS, there was speculation that *G. lamblia* might be an important pathogen in this group of individuals. However, to date, clinical findings do not seem to confirm this possibility (29, 30).

Routine stool examinations are normally recommended for the recovery and identification of intestinal protozoa. However, because *G. lamblia* organisms are attached so securely to the mucosa by means of the sucking disk, a series of even five or six stools may be examined without recovery of the organisms. The organisms also tend to be passed in the stool on a cyclical basis. The Entero-Test capsule can be helpful in recovering the organisms, as can the duodenal aspirate. Although cysts can often be identified on the wet stool preparation, many infections may be missed without the examination of a permanent stained smear (31). Material from the string test (Entero-Test) or mucus from a duodenal aspirate should be examined as a wet preparation for motility; however, motility may be represented by nothing more than a slight flutter of the flagella because the organism will be caught up in the mucus. After diagnosis, the rest of the positive material can be preserved as a permanent stain.

Immunoassays to detect *Giardia* antigen in feces have also been developed (24–26, 32). The enzyme-linked immunosorbent assays are more sensitive than microscopic examinations. Fluorescence methods involving monoclonal antibodies have also proven to be extremely sensitive and specific in detecting *G. lamblia* in fecal specimens, as have the rapid cartridges. All of the fecal immunoassays for *Giardia* tend to have comparable sensitivities and specificities; choice often depends on which method fits into the laboratory work flow. Automated molecular PCR panels, including some parasites such as *Giardia*, *Cryptosporidium*, *Cyclospora*, and *Entamoeba histolytica*, are also available. Other potential targets include *Blastocystis* spp., *Dientamoeba fragilis*, and the microsporidia.

Unfortunately, serodiagnostic procedures for giardiasis do not yet fulfill the criteria necessary for wide clinical use, particularly since they may indicate either past or present infection. In contrast, the detection of antigen in stool or visual identification of organisms with monoclonal antibody reagents indicates current infection. With the increase in the prevalence of *Giardia* infections and awareness of particular situations such as nursery school settings, perhaps additional detection assays will emerge as rapid and reliable immunodiagnostic procedures. Because giardiasis may not produce any symptoms at all, demonstration of the organism in symptomatic patients may not rule out other possibilities such as peptic ulcer, celiac disease of some other etiology, strongyloidiasis, and possibly carcinoma.

If giardiasis is diagnosed, the patient should be treated. Giardiasis can be eliminated from most patients by metronidazole (33). Tinidazole has also been used and has proven to be more effective than metronidazole as a single dose (33).

In the absence of a parasitologic diagnosis, the treatment of suspected giardiasis is a common question with no clear-cut answer. The approach depends on the alternatives and the degree of suspicion of giardiasis, both of which will vary among patients and physicians. However, it is not recommended that treatment be given without a good parasitologic workup, particularly since relief of symptoms does not allow a retrospective diagnosis of giardiasis; the most commonly used drug, metronidazole, also targets organisms other than *G. lamblia*.

The third question involves treatment of a patient who is asymptomatic. Generally, it is recommended that all cases of proven giardiasis be treated because the infection may cause subclinical malabsorption, symptoms are often periodic and may appear later, and a carrier is a potential source of infection for others (33).

G. lamblia is transmitted by ingestion of viable cysts. Although contaminated food or drink may be the source, intimate contact with an infected individual may also provide the infection mechanism. This organism tends to be found more frequently in children or in groups that live in close quarters (4, 34–40). Often, there are outbreaks due to poor sanitation facilities or breakdowns in sanitation as evidenced by infections in travelers and campers. There is also an increase in the prevalence of giardiasis in the male homosexual population, probably as a result of anal or oral sexual practices.

During the past few years, this infection has received much publicity. With increased travel, there has been a definite increase in symptomatic giardiasis within the United States. Various surveys show infection rates of 2 to 15% in various parts of the world.

Entamoeba histolytica

Although *E. histolytica* is not generally associated with health care-associated infections, reports indicate that transmission is certainly possible (41). Istre et al. reported on 36 cases of amebiasis during a 30-month period in patients who had received colonic irrigation therapy (42).

Epidemiologic studies implicated the colonic irrigation machine; testing indicated that after routine cleaning, the machine was contaminated with *E. histolytica*. Patients at greatest risk were those who received colonic irrigation immediately after a patient with amebiasis and bloody diarrhea. Evidence supports the possibility of transmission if endoscopic equipment is not thoroughly cleaned and disinfected after use in a patient infected with *E. histolytica*.

Since *E. histolytica* can easily be confused with macrophages (trophozoites) and polymorphonuclear leukocytes (cysts) found in stool, it is important to recognize the possibility of false-positive results (in which other protozoa or human cells are confused with this organism). Recognizing the possibility of error is particularly important in a potential "outbreak" situation. These types of diagnostic errors, whereby large numbers of patients were diagnosed with amebiasis, have been documented; in one report, on review, 34 of 36 patients who were diagnosed with amebiasis were found to be negative for *E. histolytica* (43).

Over the years, some workers came to believe that what was called *E. histolytica* was really two separate species of *Entamoeba*, one being pathogenic and causing invasive disease (*E. histolytica*) and the other being nonpathogenic and causing mild or asymptomatic infections (*E. dispar*). Although this was controversial, current knowledge indicates that pathogenic *E. histolytica* is the etiologic agent of amebic colitis and extraintestinal abscesses while nonpathogenic *E. dispar* causes no intestinal symptoms and is not invasive in humans (44). Unfortunately, with the exception of some newly introduced immunoassay procedures, most laboratories will continue to use routine methods for the identification of organisms in the *Entamoeba* genus; unless the trophozoites contain ingested erythrocytes, identification to the species level is not possible unless confirmed using molecular methods or the fecal immunoassay specifically for *E. histolytica*. The organisms should be reported as *E. histolytica/E. dispar* with appropriate reporting to public health agencies. In these cases, the approach to the patient will be based on clinical findings and physician decisions regarding therapy. Diagnostic reagents for confirmation of true pathogenic *E. histolytica* and for the *E. histolytica/E. dispar* group are commercially available. As with *Cryptosporidium* spp. and *Giardia lamblia*, automated molecular PCR panels also include *E. histolytica*.

Microsporidia

The evolutionary relationship of microsporidia to fungi, either as sister group or internal branch within the fungi, has been extensively debated over the years. The main difficulties for inferring microsporidian relationships stem from their highly divergent molecular sequences and the challenges these represent for reliable phylogenetic analyses. It appears that that microsporidia share a particularly close evolutionary relationship with the zygomycetes; however, actual classification of the microsporidia remains undecided (45).

Although the epidemiology of the microsporidia has not yet been thoroughly defined, it is highly likely that the spores shed in human clinical specimens (stool, urine, sputum, etc.) are immediately infectious. These infectious spores can be found in hospital and laboratory environments and are associated primarily with clinical specimens from immunocompromised patients, such as those with AIDS or transplant recipients. Precautions should be used when handling body fluids from these patients. Microsporidial infections in all body sites have been documented; this fact needs to be considered when determining the housing and care of the patient (28). Precautions would be very similar to those used for patients diagnosed with disseminated cryptosporidiosis, in which infectious organisms could be found in multiple clinical specimens.

Microsporidia are becoming more widely recognized as causative agents of ocular, gastrointestinal, renal, pulmonary, and sinus diseases in both immunocompetent and immunosuppressed patients. Ocular microsporidiosis can be isolated or may present as part of systemic infection. Recent reports indicate an increasing number of cases of ocular microsporidiosis in immunocompetent individuals. The ocular infection occurs mainly in two forms: keratoconjunctivitis, which is seen in immunocompromised individuals, and stromal keratitis, seen in immunocompetent individuals. The ocular cases which present with superficial keratitis in AIDS patients differ from the cases seen in immunocompetent individuals, which present mainly as deep stromal keratitis. Again, these ocular specimens will most likely contain infectious spores; thus handling precautions are highly recommended.

Specific infection control guidelines for inpatients with microsporidiosis have yet to be developed, but information suggests that all body fluids that may contain infectious spores should be handled accordingly. Standard precautions used within the institution and laboratory environments should be sufficient to prevent accidental infection with microsporidial spores.

Cystoisospora (*Isospora*) *belli*

C. belli is thought to be the only species of *Cystoisospora* that infects humans, and no other reservoir hosts are recognized for this infection. Transmission is through ingestion of water or food contaminated with mature, sporulated oocysts. Sexual transmission by direct oral contact with the anus or perineum has also been postulated, although this mode of transmission is probably much less common.

The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist; oocysts usually mature within 48 h following stool evacuation and are then infectious. Although it is not considered a "common" parasite, occasional positive specimens are seen in many laboratories. Since transmission is through the ingestion of infective oocysts, there is certainly the possibility that this parasite could be transmitted within the laboratory or in the patient care setting, particularly if the laboratory was involved in sporulation studies leading to infective oocysts.

Examination of a fecal specimen for the oocysts is recommended. However, wet-preparation examination of fresh material either as the direct smear or as concentrated material is recommended rather than the permanent stained smear. The oocysts are very pale and transparent and can easily be overlooked. They can also be very difficult to see if the concentration sediment is from polyvinyl alcohol-preserved stool. The light level should be reduced, and additional contrast should be obtained with the microscope for optimal examination conditions. It is also quite possible to have a positive biopsy specimen but not recover the oocysts in the stool because of the small numbers of organisms present. These organisms are acid fast and can also be demonstrated by using auramine-rhodamine stains.

Hymenolepis nana

Although *H. nana* can be transmitted by the eggs and does not require an intermediate host to complete its life cycle, the significance of this infection in the health care setting is unknown. However, it is important to remember that the eggs passed in the stool are mature and infective for patients and health care workers who may come in contact with fresh fecal material. Certainly, these infections have been documented in food handlers in many areas throughout the world.

Taenia solium

Health care-associated transmission of the infective eggs of *Taenia solium*, causing cysticercosis, has not been documented. However, an individual infected with adult *T. solium* and passing eggs could certainly serve as a potential reservoir for serious transmission potential. It is important to remember that the eggs passed in the stool are mature and infective for patients and health care workers. This is true even when proglottids are submitted to the laboratory in preservative; the eggs within the uterine branches will remain viable and infectious. Thus, handling of fresh fecal material requires careful adherence to precautions, hopefully eliminating the possibility of transmission of infectious eggs.

Cysticercosis is endemic in most of Latin America, sub-Saharan Africa, and vast parts of Asia, including the Indian subcontinent. It has also been increasingly diagnosed in developed countries because of migration of people from endemic zones and exposure in travelers. Humans get infected by the fecal-oral route, most often from a direct contact with an asymptomatic *T. solium* carrier (46).

Blood and Tissue Infections

Plasmodium spp.

Health care-associated malaria represents person-to-person transmission of parasite-infected erythrocytes through blood transfusion, needlestick injury, improper use of blood glucometers, multidose heparin vials, organ transplantation, contaminated catheters that deliver contrast medium, or rarely, open wounds. Worldwide, transfusion malaria is the most common and significant health care-related parasitic infection, although it is rare within the United States (14, 47, 48). These infections have occurred after transfusion not only of whole blood but also of white blood cells, fresh plasma, and platelets (14). Malaria infections have also originated through renal transplants (14). It is interesting that malaria infections have been transmitted from recipient to donor in a person-to-person transfusion situation in which backflow of blood has been a problem (47). Most cases of posttransfusion malaria may go unsuspected, with an incubation period of up to 4 weeks. Donors implicated in transfusion-transmitted malaria tend to be "semi-immune" with a very low parasite load; therefore, detection using antigens or PCR may not guarantee identification of the infected donor. Often the infectious dose from these donors may be 1 to 10 parasites in a unit of blood (49). All species of *Plasmodium* can survive and remain potentially invasive for 7 days in preserved blood and up to 2 years in frozen blood. The source of infection may be not only whole blood, but also the blood products derived from infected donors, such as concentrated red blood cells, white blood cells, platelets and cryoprecipitate.

In one situation, *Plasmodium falciparum* malaria was diagnosed in two patients who had been treated in the same emergency room. The two patients were in the room for 9 hours, positioned 7 m apart, and were given several emergency diagnostic and therapeutic treatments. Case 1 had pulmonary tuberculosis; he received intravenous fluid via a peripheral vein, his gastrostomy incision site was disinfected, and the dressing was changed. Intervention for Case 2 consisted of blood examination, central vein catheter insertion, intubation, mechanical ventilation, and cardiac massage. Case 2 died of falciparum malaria on the day of emergency room admission; Case 1 died of falciparum malaria 3 weeks after admission. Subsequent interviews revealed that three nurses and one emergency worker handled both patients. To confirm this possible health care-associated transmission, sequence analysis of parasites was performed. PCR and sequencing confirmed that the two patients were infected with the same organism. Case 1 had not visited an area of malaria endemicity for over 30 years; he had previously received blood transfusions, but it was confirmed that the donor's blood was malaria-free. There were no mosquitoes inside or outside the hospital. Also, natural transmission of malaria by a mosquito was not possible, because, aside from the inhospitable environmental conditions of the hospital for *Anopheles*, the time period was too short. Unfortunately, no foci of exposure to contaminated intravenous solutions, catheters, syringes, or other devices were identified, since the investigation took place 20 days after the assumption of nosocomial transmission (50).

Malaria induced via contaminated needles shared among drug addicts has been well known for many years. Also, patient-to-staff malaria transmission via needlestick injury has been documented, with most cases involving nurses or physicians who were exposed during venipuncture or preparation of blood smears (14).

In another case, transmission of *P. falciparum* was reported and confirmed by molecular genotyping from improper use of saline flush syringes in a tertiary care hospital in the United States. Although hospital policy had strict guidelines for using multidose devices and did not allow the reuse of single-use devices, interviews of seven nurses, including those who cared for the two patients, showed that two nurses admitted reusing saline flushes on the same patient, and four nurses had observed saline flushes being reused in the same patient unit (51).

An outbreak of chloroquine-resistant *P. falciparum*, in which a multidose heparin vial was implicated as the source of infection, was reported from Venezuela. The vial was apparently contaminated by the blood of a parenteral drug abuser who had asymptomatic malaria and who had received 2 days of intravenous fluid therapy (52).

In three cases involving suspected malaria transmission by renal transplantation, the donors were strongly seropositive or had a positive blood smear. However, all three patients were from areas where malaria was endemic, and the stress of surgery and immune system suppression could have also stimulated recrudescence of the infection (14). Transmission of malaria has also been documented through liver transplantation; unfortunately, the recipient died. Based on therapeutic toxicity for *Plasmodium* spp., the use of organs from donors infected with malaria carries risks that may limit transplantation from such donors (53).

Babesia spp.

The majority of cases in the United States are caused by *Babesia microti* and occur in the Northeast and upper Midwest. A small number of infections caused by *Babesia duncani* and *B. duncani*-like organisms have been reported on the West Coast from California to Washington State. Additionally, *Babesia divergens*-like organisms have been reported in Kentucky, Missouri, and Washington State. In Europe, almost all reported cases have been due to *B. divergens*, and a few have been caused by the EU1 species, now called *Babesia venatorum*. Sporadic cases of babesiosis have also been reported in Asia, Africa, Australia, and South America. As the frequency of clinical cases has risen, there has been an associated increase in transfusion-transmitted *Babesia*, making babesiosis the most frequent transfusion-transmitted infection with approximately 162 cases reported since 1980 and 12 associated fatalities in the period 2005–2008 (54). While the current strategy of blood screening via donor questionnaires permanently excludes prospective blood donors with a history of babesiosis, it appears to be of limited value because infected blood donors usually experience asymptomatic infection or remain infectious long after symptoms have resolved.

Transmission of babesiosis through routine blood transfusion, frozen-thawed blood, and platelets has also been documented (14, 55). Although needlestick or open-wound transmission of *Babesia* spp. has not been reported, it is certainly possible, particularly in the health care setting. Refrigeration of *B. divergens* results in parasite morphology changes and a decrease in parasite numbers. However, there are sufficient parasites that survive 31 days of storage at 4°C and yield high end-point parasitemia. The risk of infection through contact with ticks is probably low.

Trypanosoma brucei gambiense and *T. brucei rhodesiense*

Although patients with African trypanosomiasis are usually symptomatic, those in the early stages of the infection may be asymptomatic and appear to be healthy potential blood donors. These types of patients are probably responsible for the rare transfusion cases reported. However, contaminated needlestick injuries and open-wound contact with contaminated blood are also potential means of transmission in the health care setting.

Trypanosoma cruzi

Although *Trypanosoma cruzi* is normally transmitted by the feces of an infected triatomid bug, transmission of the organism via blood transfusion is the second most common means of acquiring the infection. In areas where infection is highly endemic, as many as 62% of blood donors are seropositive for *T. cruzi* antibody (56). One study from Los Angeles County of 1,027 consecutive blood donations found 2.4% to be seropositive for *T. cruzi* antibody by an indirect fluorescent-antibody assay (57). In another study, lookback analysis was able to identify the first two cases of probable transfusion-transmitted *T. cruzi* infection since implementation of the national screening program, which increases the total number of reported cases in the United States to eight. Considering this information, concerns regarding the safety of the blood supply are reasonable and understandable (58). Transmission via kidney transplants has also been well documented (59).

The entry of large numbers of immigrants from Central America has contributed to growing concerns, particularly with respect to transfusion-related transmission of *T. cruzi* in areas of the world where the infection is not endemic or, if endemic, has not been a problem. Since there are triatomid bugs capable of becoming infected with *T. cruzi* within the southwestern United States, the presence of more people with latent infections also presents some concerns (60). Currently, it is estimated that 300,000 people with Chagas' disease live within the United States, and one in 354,000 blood donors might have acquired *T. cruzi* within the United States (60). In another reference, it is estimated that as of 2009, 409,000 United States residents have Chagas' disease; over a period of 20+ years, as many as 300 patients may have contracted Chagas' disease through transplantation (81).

The reported rate of confirmed *T. cruzi* infection in volunteer U.S. blood donors is approximately 1 in 27,500, so with about 30,000 tissue donors annually in the United States, tissue banks should expect to encounter one or two *T. cruzi*-infected tissue donors each year. Solid organ donation of the heart, liver, kidney and kidney/pancreas combination, lungs, bone marrow, and cord blood from infected donors has resulted in transmission to recipients in both endemic and nonendemic countries. Transmission of *T. cruzi* through donor kidneys has been documented in Latin America, where serologic screening of organ donors and recipients is not standard practice. Three cases of recipient infection from a single donor from Central America have been reported in the United States; the cases involved kidney and pancreas, kidney alone, and liver. One of the recipients died of myocarditis (53).

Tissue donation presents a unique situation with the possibility of parasites infecting blood and tissue in the deceased donor prior to tissue recovery, chemical processing, irradiation, or lyophilization during tissue processing, and short-term storage at refrigerated temperatures and long-term storage at frozen temperatures. The ability of *T. cruzi* to survive long periods of storage at +4 and −80°C suggests that *T. cruzi*-infected tissues stored under these conditions are potentially infectious. Viable parasites capable of infecting cells in culture were present at 1 year at −80°C frozen storage even in the absence of cryoprotectant.

In the United States, nine cases of *T. cruzi* transmission from solid organ donation have been documented. Eight reported cases of *T. cruzi* transmission via blood transfusion have been reported; blood donor screening was implemented in the United States in 2007 (61). Current Food and Drug Administration guidance specifies that all blood donors must be asked about a history of Chagas' disease. However, it has been determined that the Chagas' history question has no real value, while testing donor units provides the best option for detection of potentially positive donors (62). Proposed testing options for transplant donors include automatic testing based on the medical patient questionnaire, testing of all recipients, testing all individuals of Latin origin, and testing of all organ donors (81).

The oral consumption of Amazonian palm berry or açaí juice, probably contaminated with triatomid bug feces on the fruit or with bugs that were accidentally crushed during the preparation process, is another possible means of *T. cruzi* transmission in some areas of the world. Without subsequent pasteurization, the organisms remain viable, especially if the juice is not frozen.

Contaminated needlestick injuries, accidental exposure to positive cultures, processing infective tissues, and open-wound contact with contaminated blood are also potential means of transmission in the health care setting (18).

Leishmania donovani

Although transfusion-induced visceral leishmaniasis has been described only rarely, like other infections mentioned above it may be underreported in areas of endemicity where confirmation of the route of infection may be difficult. Certainly, exposure to contaminated blood or animals would represent a potential source of infection.

Toxoplasma gondii

It is well documented that immunosuppression (advanced HIV disease, medications used in cancer therapy or organ transplantation) can result in disease reactivation and severe morbidity or mortality. Health care-associated infection with *Toxoplasma gondii*, primarily in cardiac or renal transplant patients, has been documented (14, 63). However, the efficacy of trimethoprim-sulfamethoxazole for the prevention of toxoplasmosis transmission dramatically reduces the risk to organ recipients from infected donors. Most serious infections in immunocompromised patients result from reactivation of a latent infection. Transmission from a patient to a health care worker via needlestick injury or exposure to blood (open wound) has not been documented.

Studies have revealed associations between *T. gondii* antibody seropositivity (indicating infection) and the presence of various psychiatric disorders in humans (e.g., schizophrenia, bipolar illness, suicide attempts, episodes of self-directed violence, and memory impairment in the elderly). Although these results are interesting and potentially indicate another reason to prevent toxoplasmosis, additional studies are needed to fully explain possible relationships between *T. gondii* infection and mental illness, and how the strain and stage of *T. gondii* (oocyst versus tissue cyst) may impact this relationship (64).

Infections with Ectoparasites

Ectoparasites of nosocomial significance include lice (head, body, pubic), scabies and other mites, and myiasis. Head and pubic lice and scabies are fairly common in many parts of the world, including the United States. These infestations are generally not serious problems within the health care setting, with the exception of scabies, which has the potential to cause large outbreaks.

Pediculus spp. and *Phthirus pubis*

Infection with lice belonging to the genus *Pediculus* are common and are usually found on one part of the body such as head, body, or pubic hair. The head louse, *Pediculus humanus capitis*, is found most often in school-age children, and nosocomial transmission is generally not a problem, with possible exceptions characterized by close patient-to-patient contact in pediatric wards or institutional settings.

Pediculus humanus corporis (body louse) is well known for its association with poverty and poor personal hygiene. Since transmission is through contact with or exchange of contaminated clothing or bedding, this does not seem to represent a large potential problem. However, the potential for transmission varies throughout the world.

Phthirus pubis (pubic louse) infestation occurs most commonly in sexually active adults, with a direct skin-to-skin transfer. It is unlikely that transmission within the health care setting would cause any problems.

Sarcoptes scabiei

Scabies is a highly contagious infestation of the itch mite, *Sarcoptes scabiei*. Symptoms are related to pruritic dermatitis, and apparently, transmission can occur through person-to-person direct skin contact or via fomites. Outbreaks of scabies in hospitals or institutions, particularly for the elderly or developmentally disabled, have been documented worldwide (65). These outbreaks are particularly severe when associated with crusted (Norwegian) scabies; such patients may be infected with thousands of mites on the skin. This infection has been linked to cellular immune deficiencies including AIDS (66). Other potential problems include a decreased awareness of itching and the use of topical corticosteroids (65). Because of the number of mites present, these patients are extremely contagious via sloughing skin, direct contact, and environmental contamination. Another potential problem is the fact that the symptoms mimic those of many other dermatologic problems. Burrows may not be evident, and hyperkeratotic, crusted, scaling, fissured plaques are present over the scalp, face, and back, with associated gross nail thickening. The number of documented reports of scabies, particularly in AIDS patients, suggests that health care-associated scabies may become more common in the future.

Missed diagnoses of scabies during emergency room visits may result in nosocomial spread and increase the unnecessary use of prophylactic treatments. Emergency room overcrowding, time constraints, and less severe illness compromise recognition of scabies in this setting. Health care workers should be alert for signs of scabies infestations under these conditions.

Although outbreaks of other mites, including the pigeon mite *Dermanyssus gallinae*, have been described, it is not clear whether these infestations are a problem. It has also been suggested that these cases may be misdiagnosed as scabies (65).

Myiasis

Myiasis refers to infestation of the body by larvae of a number of fly species. These cases occur when flies are attracted to patient blood or body tissue fluids and deposit eggs into an open wound or patient body opening such as the nose or ear. Often, these patients are not totally conscious, or they may be debilitated or immobile. It is generally thought that these cases are underreported for a number of reasons, some of which are related to institutional risk management decisions. A study from Brisbane, Australia, reported 14 infestations, 6 of which were health care-associated, during a period of 17 months (67). Almost all reported cases of nosocomial myiasis (nasal, submandibular, oral, ulcer, unbroken skin, wound, vaginal, nasotracheal, conjunctival, surgical incision) have been in patients with underlying disease or trauma.

Ophthalmomyiasis, caused by infestation of the eye by dipterous fly larvae, can result in symptoms ranging from minor irritation to blindness, disfigurement, or death. Infestations with the sheep botfly, *Oestrus ovis*, are normally associated with normal hosts, while infestations with others, including *Cochliomyia hominivorax*, seem to occur more commonly in compromised hosts (68).

Infections in the Pediatric Patient

Specific infections that can be acquired in the pediatric patient include those caused by *Cryptosporidium* spp., *G. lamblia*, *P. humanus capitis*, and *S. scabiei*. Although less is known about microsporidia, the infective spores could be transmitted in a hospital setting, particularly if spores are present in stool specimens, urine, or other body fluids from immunocompromised or immunocompetent patients.

Cryptosporidium spp.

Cryptosporidium infection is discussed above in the section on gastrointestinal infections. It is also important to remember than this organism has been isolated from other body sites, particularly in severely immunocompromised patients. Cryptosporidiosis has also been linked to infections in nursery schools and should certainly be considered in this setting.

Giardia lamblia

Infection has been well documented in the pediatric population. Giardiasis is most frequently associated with nursery school outbreaks and is thoroughly discussed above in the section on gastrointestinal infections.

Pediculus humanus capitis

Although pediculosis is a well-known problem among young children, lice are not as likely to be spread to other patients except in chronic ambulatory-care situations such as psychiatric wards or pediatric ward playrooms. Under these circumstances, patients should be screened prior to admission.

Sarcoptes scabiei

Prevalence of the itch mite infection, or scabies, varies among young children; sexually active adolescents and adults are also susceptible to infection. The small size of the mite contributes to the problem and to possible transmission to medical staff and other patients. Although intimate contact is considered the most likely mode of transmission, fomite transmission is an alternative method, particularly with crusted scabies. It is generally recommended that if two or more patients and one or more staff members exhibit the symptoms of scabies (pruritus with or without crusting or scaling) or have positive skin scrapings, total treatment should be given. It is also important that posttreatment surveillance and testing be continued for at least 3 months.

Infections in the Compromised Patient

Compromised patients have poor host defenses, usually for one of three reasons: underlying disease (immunosuppression or immunodeficiencies in either the cellular or humoral mechanisms of the immune system or both), therapy for disease (chemotherapy, radiation), or hospital-based procedures and contact with infected persons (catheterization, broad-spectrum antibiotics, nebulizers, inhalation procedures, person-to-person contact, food sources, contaminated equipment, etc.).

In addition to *Cryptosporidium*, *E. histolytica*, *G. lamblia*, and microsporidia as mentioned above, *S. stercoralis*, free-living amebae, *Toxoplasma*, *Trypanosoma* spp., and *Leishmania* spp. cause severe disease in the compromised patient and can be transmitted in the hospital setting.

S. stercoralis has become extremely important, particularly with respect to compromised patients (69) and military personnel and travelers who may have been in an area of endemic infection many years before (70). More than 30 to 40 years after acquisition of the original infection, persistent undiagnosed disease can be found in these individuals. If, for any reason, they become immunocompromised, the result can be disseminated disease leading to the hyperinfection syndrome and death (71–73).

The life cycle of *S. stercoralis* is one of the more complex parasite cycles. Human infection is acquired by skin penetration of the filariform larvae (infective larvae) from the soil. After penetration of the skin, the larvae are carried via the cutaneous blood vessels to the lungs, where they break out of the pulmonary capillaries into the alveoli. They then migrate via the respiratory tree to the trachea and pharynx, are swallowed, and enter the mucosa in the duodenum and upper jejunum. Development usually takes about 2 weeks, after which the females begin egg production. The eggs are oval and thin shelled and measure 50 to 58 μm by 30 to 34 μm (generally a bit smaller than hookworm eggs). The eggs usually hatch, and the rhabditiform larvae (noninfective larvae) pass out of the intestinal tract in the feces. These larvae are passed out onto the soil in the feces and develop into free-living male and female worms, eventually producing infective filariform larvae (egg, noninfective larvae, and infective larvae). In temperate climates, the free-living male and female worms do not develop; however, the rhabditiform larvae that pass out in the stool develop into the filariform (infective) larvae, which are then ready to infect the next host through skin penetration.

In situations in which autoinfection occurs, some of the rhabditiform larvae that are within the intestine develop into the filariform larvae while passing through the bowel. These larvae can then reinfect the host by (i) invading the intestinal mucosa, traveling via the portal system to the lungs, and returning to the intestine or (ii) being passed out in the feces and penetrating the host on reaching the perianal or perineal skin.

It is generally accepted that the life cycle presented above is correct; the skin-penetrating larvae travel from the skin to the intestine via an obligatory route that includes the blood, lungs, trachea, and upper gastrointestinal tract (the standard pulmonary route). It is also assumed that following autoinfection, the larvae follow the same route to return to the duodenum, where they mature. However, review information and data from Schad et al. (74) indicate that the pulmonary route is just one of several possible pathways to the duodenum, regardless of whether the larvae penetrate the skin or the intestine. In spite of some caveats, these studies definitely challenge the current accepted understanding of the life cycle. The pulmonary migration route for all skin-penetrating nematodes may not be as universally applicable as was once thought.

As judged from the life cycle, the three areas of primary involvement are the skin (cutaneous), the lungs (pulmonary), and the intestinal mucosa (intestinal). Initial skin penetration usually causes very little reaction, although there may be some pruritus and erythema if the number of penetrating larvae is large. With repeated infections, the patient may mount an allergic response that will prevent the parasite from completing the life cycle. The larvae may be limited to skin migration or larva migrans.

Larval migration through the lungs may stimulate symptoms, depending on how many larvae are present and the intensity of the host immune response. Some patients are asymptomatic, whereas others present with pneumonia. With a heavy infective dose or in the hyperinfection syndrome, individuals often develop cough, shortness of breath, wheezing, fever, and transient pulmonary infiltrates (Loeffler's syndrome). Cases in which the larvae could even be found in the sputum have been reported (75, 76).

In heavy infections, the intestinal mucosa may be severely damaged with sloughing of tissue, although this type of damage is unusual. The symptoms may mimic peptic ulcer with abdominal pain, which may be localized in the right upper quadrant. Radiographic findings may mimic Crohn's disease of the proximal small intestine (77). In an immunocompetent patient, there is a leukocytosis with a peripheral eosinophilia of 50 to 75%, while in patients with chronic infections, the eosinophilia may be much lower. Some of these chronic infections have lasted over 30 years as a result of the autoinfective capability of the larvae. One case of chronic strongyloidiasis persisted for approximately 65 years (78).

Autoinfection is probably the mechanism responsible for long-term infections that persist for years after the person has left the area of endemic infection. The parasite and host reach a status quo so that neither suffers any serious damage. If for any reason this equilibrium is disturbed and the individual becomes immunosuppressed, the infection proliferates, with large numbers of larvae being produced and found in every tissue of the body. Several conditions, including the increased use of immunosuppressive therapy, predispose an individual to the hyperinfection syndrome. In addition to suffering tissue damage from the migrating larvae, the patient may die from sepsis, primarily as a result of the intestinal flora. Other causes of death include peritonitis, brain damage, and respiratory failure. Few cases of reactive arthritis have been reported, but the number of cases may be underestimated. A case of reactive arthritis combined with uveitis associated with a long-standing, heavy *Strongyloides* infection was detected in a 32-year-old patient positive for human T-cell leukemia virus type 1. Treatment with thiabendazole and ivermectin resulted in rapid improvement.

Confirmation of the infection depends on recovery and identification of the adult worms, larvae, or eggs from the stool, duodenal material, or sputum. Fecal specimens may not be positive, even after performance of the routine ova and parasite examination, including the concentration procedure. The number of larvae found in the stool also varies from day to day.

Some reports indicate that sampling of duodenal contents may recover larvae if stools are negative, while others indicate that duodenal sampling may not be effective. Special techniques that have been recommended for the recovery of duodenal contents include the Entero-Test capsule. There are also special concentration techniques (Baermann) and methods for larva culture (Harada-Mori, petri dish). These techniques provide a means of larval recovery from large amounts of stool material, and often the filariform larvae are recovered. To differentiate the *Strongyloides* filariform larvae from those of hookworm, one should examine the tail under a microscope. *Strongyloides* filariform larvae have a slit in the tail, while hookworm filariform larvae have a pointed tail.

Agar plate cultures are also possible; stool is placed onto agar plates, and the plates are sealed to prevent accidental infections and then held for 2 days at room temperature. As the larvae crawl over the agar, they carry bacteria with them, thus creating visible tracks over the agar. The plates are examined under the microscope for confirmation of larvae, the surface of the agar is then washed with 10% formalin, and larval identification is confirmed via wet examination of the sediment from the formalin washings.

Debilitated or compromised patients should always be suspected of having strongyloidiasis, particularly if there are unexplained bouts of diarrhea and abdominal pain, repeated episodes of sepsis and/or meningitis with intestinal bacteria, or unexplained eosinophilia. However, a recent comparative study of the occurrence of *Strongyloides* infection in 554 AIDS and 142 non-AIDS patients demonstrated similar prevalences of infection in the two groups, thus indicating no significant statistical differences (79).

At present, the recommended drug of choice is ivermectin or albendazole. In some cases, additional drugs or repeated therapy may be necessary. Patients with the hyperinfection syndrome should be hospitalized during therapy for proper monitoring.

Contact with contaminated infective soil, feces, or surface water should be avoided. Individuals found to have the infection should be treated. *All patients who are going to receive immunosuppressive drugs should be screened for strongyloidiasis before therapy.*

Laboratory Infections

Safety precautions tend to be emphasized in clinical laboratories, particularly microbiology laboratories. However, general safety regulations pertaining to laboratory coats, hand washing, and use of disinfectants, as well as to prohibitions of eating, drinking, and smoking, fail to address the specifics of possible parasite transmission.

In laboratories where extensive diagnostic testing is performed, exposure to possible infectious parasites may result from the handling of specimens, drawing of blood, various types of concentration procedures, organism cultures, and animal inoculation studies. Relevant parasites and their possible routes of infection are presented in Tables 31.2 and 31.3. Information on resistance to antiseptics and disinfectants can be found in Table 31.4.

Intestinal Protozoa

Intestinal protozoa, such as *G. lamblia*, *Dientamoeba fragilis*, *E. histolytica*, *Blastocystis* spp., microsporidia, and the coccidia, particularly *Cryptosporidium* spp., cause human disease, and the protozoan cysts, oocysts, and spores can be accidentally transmitted from one person to another. The cysts, oocysts, and spores are resistant to many disinfectants. Of those currently available, ozone is the most effective protozoan cysticide, followed by chlorine dioxide, iodine, and free chlorine, all of which appear to be more effective than the chloramines. Cyst forms, including oocysts, are the most resistant to chemical disinfection. Although the exact mechanisms of resistance are not thoroughly understood, these resistant stages probably take up fewer disinfectant molecules from solution than do stages like the trophozoite (Table 31.4).

Relatively few cases of laboratory-acquired infections with intestinal protozoa have been reported; however, a few cases have been detected. A worker who "checked in several hundred stool specimens, stamping numbers and dates on report cards, many of which had been contaminated from leaky containers," became infected with *G. lamblia* (18). The infection was confirmed by finding the flagellates in the stool. A laboratory technician who examined numerous stool specimens from a patient infected with *C. belli* became ill about a week later, and *C. belli* was confirmed to be present in his stool. While infecting a rabbit using a capsule containing about 400 *Cystoisospora* oocysts, two researchers were sprayed in their faces with droplets when the rabbit regurgitated the material and vigorously shook its head; both individuals became ill about 2 weeks later (18).

Free-Living Amebae

A number of studies have investigated the responses of trophozoites and cysts of *Acanthamoeba* spp. to disinfectants in various solutions used to disinfect contact lenses; infections with these organisms have led to serious eye infections, particularly keratitis. These infections can be very difficult to treat if not recognized early, and eradication of the free-living amebae is also very difficult. These protozoa are capable of forming biofilms on contact lenses and could adversely affect the effectiveness of chemical agents used for disinfection. For those laboratories that routinely culture specimens for the free-living amebae, handling of the specimens and/or culture material represents a potential source of transmission and infection.

Plasmodium spp.

In a series of 34 cases of laboratory-acquired malaria, 15 were due to *P. falciparum*, 10 were due to *P. cynomolgi*, and 9 were due to *P. vivax*. The majority of cases were reported from the United States and Europe, with 19 being vector borne, 10 being parenteral, and 5 occurring through a break in the skin. All patients were symptomatic (18, 80).

It has also been documented that research workers can be accidentally infected from a mosquito, possibly one that escaped from a mosquito colony. Strict containment measures should be in place. Another possible route of infection involves mosquito dissection, during which infective sporozoites could be accidentally injected.

Trypanosoma brucei gambiense and *T. brucei rhodesiense*

Cases of laboratory-acquired African trypanosomiasis can result from contact with blood or tissue from infected persons or animals. Six laboratory-acquired cases have been caused by *T. brucei gambiense*, and two have been

TABLE 31.2 Possible parasite transmission in a health care setting (laboratory)

Organisms[a]	**Mode of transmission**	**Comments**
Protozoa (intestinal)		
Entamoeba histolytica[b,c] (C) *Entamoeba dispar* (C) *Entamoeba coli* (C) *Entamoeba hartmanni* (C) *Endolimax nana* (C) *Iodamoeba bütschlii* (C) *Blastocystis* spp.[b] (C) Free-living amebae *Giardia lamblia*[b,c] (C) *Dientamoeba fragilis*[b] (T) *Cryptosporidium* spp.[b,c] (O) *Cyclospora cayetanensis*[b] (O) *Cystoisospora belli*[b,c] (O) Microsporidia[b] (S)	Accidental ingestion of infective cysts, trophozoites, oocysts, or spores in food or water contaminated with fecal material; also, direct transfer of stool material via fomites (fecal-oral transmission) Contamination of hands, eyes when handling free-living ameba cultures; inhalation of infectious aerosols; splashes to eyes or mucous membranes	Transmission becomes more likely when fresh stool specimens are being processed and examined; submission of fecal specimens in preservatives or fixatives would decrease risks. Wearing gloves, using capped centrifuge tubes, and working in biological safety cabinet would decrease the risk of acquiring *Cryptosporidium* infections. *Not recommended:* use of potassium dichromate as collection fluid and use of sugar-flotation on fresh stool. Immunocompromised employees must be educated regarding risk factors; they may elect not to work with certain organisms or perform certain procedures.
Helminths (intestinal)		
Enterobius vermicularis (E)[c]	Inhalation or ingestion of infective eggs	Very common in children, asymptomatic
Strongyloides stercoralis (IL)[c]	Skin penetration of infective larvae from stool material	Exposure would be very possible or even likely when working with stool cultures or concentrates for larva recovery.
Hymenolepis nana (E)	Ingestion of infective eggs (fecal-oral)	Ingestion of infective eggs can lead to the adult worm in humans.
Taenia solium (E)	Inhalation or ingestion of infective eggs (could lead to cysticercosis)	Exposure very likely when working with gravid proglottids (ink injection for identification of worm to species level).
Blood and tissue protozoa		
Leishmania spp.[c]	Direct contact or inoculation of infectious material from patient lesion; accidental inoculation of material from culture or animal inoculation studies	Culture forms and organisms from hamster would be infectious.
Trypanosoma spp.[c]	Same as for *Leishmania* spp.; also handling arthropod vectors	Cultures and special concentration techniques would represent possible means of exposure.
Plasmodium spp.[c,d]	Accidental inoculation could transmit any of the five species; mosquito transmission	Blood and culture material should always be handled carefully (avoid open cuts, etc.).
Toxoplasma gondii[c]	Inhalation or ingestion of oocysts in cat feces (veterinary situation); accidental inoculation of tachyzoites from culture, tube of blood, or animal isolation (mouse peritoneal cavity); needles, spills, mucous membrane contamination; tissue culture work	Although many people already have antibodies to *T. gondii*, indicating past exposure, there have been documented laboratory accidents in which the individual became ill because of the large infecting dose.
Ectoparasites		
Pediculus spp.	Specimens submitted on hair could be easily transmitted in the laboratory.	Careful handling and fixation of the arthropods would prevent any potential problems with transmission.
Sarcoptes scabiei	Transmission via skin scraping, etc., would be possible but not that likely.	Careful handling and preparation of specimens with KOH would tend to prevent any problems.
Dipterous fly larvae (myiasis)	Transmission could occur anywhere.	Protection from flies would solve the potential problem.

[a] C, cyst; T, trophozoite; O, oocyst; IL, infective larvae; E, egg; S, spore.

[b] Potentially pathogenic intestinal protozoa.

[c] Laboratory infections documented: *Leishmania* spp., *Trypanosoma cruzi*, *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense*.

[d] Mosquito transmission.

TABLE 31.3 Potential exposures to laboratory-acquired parasitic infections[a]

- Parenteral or aerosolization
  - Recapping a needle
  - Removing a needle from the syringe
  - Leaving a needle on the counter, point up
  - Dropping a syringe
  - Breaking hematocrit tube while pressing the end into clay
  - Performing venipuncture on agitated patient
  - Sudden animal movement during an inoculation procedure
  - Creating aerosols during tapeworm proglottid injection
  - Creating aerosols while working with cultures (blood parasites, free-living amebae)
  - Centrifugation
  - Blenders, shakers, sonicators, other mixing instruments
  - Pouring and decanting fluids
  - Spillage of infectious material
  - Lyophilization and filtration under vacuum
  - Egg inoculation and harvesting
  - Animal and insect bites and scratches
  - Injecting animals
- Animal or vector bites
  - Being bitten by an infected animal (mouse, hamster)
  - Being bitten by infected mosquito (mosquito colony)
- Skin exposure
  - Not wearing gloves during procedure
  - Not wearing laboratory coat (closed sleeves, closed front over clothes)
  - Accidentally touching face or eyes during handling of infectious materials
  - Exposing of eyes, nose, and mouth to potential aerosols
- Ingestion
  - Mouth pipetting
  - Being sprayed with inoculum droplets from coughing or regurgitating animal
  - Contaminated articles or fingers placed in mouith
  - Consumption of food in workplace
- Other reasons for potential exposures
  - Working in disorganized laboratory bench setting
  - Working too fast
  - Not receiving proper training
  - Assuming that the agent is not infectious to humans
  - Assuming that the agent(s) is no longer viable
  - Using defective equipment

[a] Adapted from a number of sources, including reference 18.

TABLE 31.4 Resistance to antiseptics and disinfectants, from most to least resistant

Type of organism	Example(s)
Prions	Creutzfeldt-Jacob disease, bovine spongiform encephalopathy
Coccidia	*Cryptosporidium* spp.
Spores	*Bacillus*, *Clostridium difficile*
Mycobacteria	*Mycobacterium tuberculosis*, *M. avium*
Cysts	*Giardia lamblia*
Small nonenveloped viruses	Poliovirus
Trophozoites	*Acanthamoeba*
Gram-negative bacteria (nonsporulating)	*Pseudomonas*
Fungi	*Candida*, *Aspergillus*
Large nonenveloped viruses	Enteroviruses, adenovirus
Gram-positive bacteria	*Staphylococcus aureus*, *Enterococcus*
Lipid-enveloped viruses	HIV, hepatitis B virus

caused by *T. brucei rhodesiense*. In these cases the route of exposure was usually parenteral, and all patients were symptomatic.

Trypanosoma cruzi

Infection can occur from exposure to the feces of infected triatomine bugs, by handling cultures or blood specimens, and possibly by inhaling aerosolized organisms. The predominant stage of the parasite in culture is the epimastigote stage; however, the infectious trypomastigotes can also be found. *T. cruzi* can infect laboratory personnel through needlestick injuries, through preexisting microabrasions of the skin, or through intact mucous membranes.

Sixty-five cases of laboratory-acquired *T. cruzi* infection have been reported, although in many cases, the actual geographic site was unknown. While the route of exposure for many of the cases was unknown, parenteral infection was identified for 11 individuals. The clinical status of the infected individuals was generally unknown; however, 24 of 47 were symptomatic, with 9 severe cases and 1 death (18, 80).

Leishmania spp.

In laboratory settings, leishmaniasis could be acquired through contact with an infected sand fly; containment measures for infected flies should be strictly followed. Transmission can also occur through contact with parasite cultures or clinical specimens from infected persons or animals (e.g., needlestick injuries or microabrasions of the skin). Although fewer parasites are found in patient blood specimens than in cultures or infected tissues, blood should be handled with care.

Twelve cases of laboratory-acquired leishmaniasis caused by six different species have been reported. Although most of the infected persons developed cutaneous leishmaniasis, sometimes with associated local lymphadenopathy, one person developed visceral leishmaniasis and one developed mucosal leishmaniasis as sequelae of cutaneous leishmaniasis. All patients were symptomatic, with two having severe disease (18).

Toxoplasma gondii

Toxoplasmosis is the most common laboratory-associated parasitic infection (48). Infection can occur through ingestion of sporulated oocysts from feline fecal specimens or through skin or mucosal contact with tachyzoites or bradyzoites in tissue or culture. All *Toxoplasma* isolates should be considered pathogenic for humans. Procedures for separating oocysts from feline feces and for infecting mice have been described; fecal concentrations should be performed before oocysts sporulate and become infectious. Laboratory equipment and glassware that have been in contact with oocysts should be sterilized, since the oocysts are not easily killed by chemical exposure. Immunocompromised-persons and *T. gondii*-seronegative women who are pregnant or might become pregnant should be counseled about the risks associated with *T. gondii* infection (e.g., central nervous system infection and congenital infection) and given the option of not working with live *T. gondii* and of not working in a laboratory in which others do so.

Of the 47 cases of laboratory-acquired toxoplasmosis, the majority were in the United States and Europe. The most common route of exposure was parenteral; however, in 10 of the cases no specific accident or route of exposure could be identified. Although 9 patients were asymptomatic, 38 were considered to be symptomatic if they had lymphadenopathy. Four individuals had encephalitis, two of whom had myocarditis, and one patient with both conditions died (18).

Specimen Handling

Another consideration involves the handling of specimens from patients with AIDS. Although the agent, HIV, is considered to be a very fragile virus that does not survive in the environment, specimen-handling precautions are advised. Some laboratories suggest that stool specimens being processed for *Cryptosporidium* (often diagnosed in AIDS patients) be handled in a biological safety cabinet and be centrifuged in capped centrifuge tubes. These types of precautions will help diminish the chances of an accidental laboratory infection.

Summary

Although there are real possibilities for health care-associated and laboratory transmission of parasites, proof of such transmission is usually very difficult to document. Depending on the area of the country and the number of endemic parasites present in the community, documentation can be particularly difficult. In areas where many organisms are endemic, it is almost impossible to differentiate between a community-acquired infection and one acquired in the work setting.

Some of the infectious agents being handled in the laboratory present a potentially more serious problem. Although many laboratories do not handle all of these agents, their personnel may still be at risk when performing certain techniques or handling certain specimens. For these reasons, it is very important that everyone become aware of possible hazards when working in the field of diagnostic medical parasitology. The information presented in Tables 31.1 through 31.3 provides specific, practical information concerning safety precautions in the laboratory.

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32

Immunology of Parasitic Infections

- Amebiasis
- Giardiasis
- Toxoplasmosis
- African trypanosomiasis
- American trypanosomiasis
- Malaria
- Helminth infections

The ability to distinguish between self and non-self is required for a functioning immune system. It is important to remember that infection with an organism does not necessarily mean diseases, since the immune system in most cases will be able to eliminate the infection before disease occurs. Disease occurs only when the infecting load is high, when the organism is quite virulent, or when immunity is compromised. Although the immune system is beneficial to the host, there can be detrimental effects as well. During an immune response, there may be local discomfort and damage to healthy tissue as a result of the toxic products produced by the immune response. Also, in some cases the immune response can be directed toward host tissues, resulting in autoimmune disease.

The immune system is made up of two interrelated parts, the innate (nonspecific) and acquired (specific) systems (1–3). The innate immune system is the first line of defense against organisms while the adaptive immune system acts as a second line of defense and also affords protection against re-exposure to the same pathogen. Each of these major parts of the immune system uses cellular and humoral components to facilitate their protective function. The innate immune system also has anatomical features that function as barriers to infection. Although these two arms of the immune system have distinct functions, there is interplay between the two. The acquired immune system requires some time to react to an invading organism, whereas the innate immune system includes defenses that are present immediately for use. In addition, the acquired immune system is antigen specific and reacts only with the organism that induced the response. In contrast, the innate system is not antigen specific and reacts equally well to a variety of organisms. Also, the acquired immune system demonstrates immunological memory; it reacts more rapidly on subsequent exposure to the same organism.

For many years, there has been extensive research in immunoparasitology directed at the identification, isolation, purification, and characterization of parasite antigens. Antigens are required for immunodiagnosis, clarification of immunopathology, quantitation of various immune responses in the host, and evaluation of potential vaccines. Antibodies to defined antigens are used for typing of reagents, the study of antigenic variability, passive immunization, and *in vitro*

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TABLE 32.1 Parasite antigens[a]

- I. Immunologic criteria
  - A. Importance of the induced immune response to the parasite or host (relevant or functional antigens versus incidental antigens)
    1. Host-protective antigens[b]: related to induction and expression of host-protective immunity
    2. Immunodiagnostic antigens: related to detection of antibodies, skin test sensitivity (both immediate or delayed), detection of circulating or urinary antigens
    3. Immunopathologic antigens[c]: related to induction and expression of immunopathology
    4. Parasite-protective antigens: related to induction and expression of parasite-protective immunity
  - B. Immunogenicity in different animals
    1. Natural antigens: infection of natural hosts or immunization of natural hosts with or without adjuvants
    2. Novel antigens (heteroantigens): immunization of unnatural hosts with or without adjuvants, or infection of nonpermissive hosts
  - C. Type of lymphocyte involved, e.g., T-cell-stimulating carrier determinant, B-cell-stimulating haptenic determinant, allergen, T_s-stimulating determinant
- II. Parasitologic criteria
  - A. Origin and location
    1. Soluble exoantigens: released from living parasites, parasitized cells, or cultured cell lines and called excretory/secretory (ES), excretory/secretory/tissue turnover (EST), or metabolic antigens
    2. Soluble somatic antigens: extracted from parasites or parasitized cells; may be surface or internal
    3. Dead parasites, fragments or secretory blebs
    4. Whole living parasites
    5. Body fluids of nematodes
    6. Cystic fluids of larval cestodes
  - B. Parasite population and life cycle
    1. Genus-, species-, strain-, and stage-specific antigens
    2. Molting antigens
- III. Biochemical criteria
  - A. Composition (protein, lipid, carbohydrate, glycoprotein, glycolipid, etc.)
  - B. Characteristics (size, chain structure, determinant number and type, etc.)
  - C. Molecule function (enzyme, metabolite, receptor, recognition structure, etc.)
  - D. Future sources (expression of cloned DNA, chemical synthesis, stimulation of anti-idiotypic antibodies)

[a] Data from reference 1.

[b] It is not the host antigens themselves but the immune responses that these antigens induce which are host protective.

[c] Immunopathologic immune responses depend on the genotype of the host and may also have beneficial effects.

inhibition studies. Without the use of defined antigens and antibodies, a thorough understanding of the immunologic aspects of the host-parasite relationship is unlikely. Table 32.1 contains information on the various categories of parasite antigens, while Table 32.2 lists various methods of antigen analysis.

TABLE 32.2 Methods of antigen analysis

- Gel precipitation techniques
  1. Double diffusion
  2. Ouchterlony
  3. Immunoelectrophoresis
  4. Autoradiography of precipitin lines
  5. Counterimmunoelectrophoresis
  6. Counterradioimmunoelectrophoresis
- Immunoprecipitation and gel analysis
  1. Anti-immunoglobulins
  2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
  3. Isoelectric focusing
  4. Nonequilibrium pH gradient electrophoresis
  5. Fluorography
- Gel overlay or transfer techniques
  1. Direct application of antiserum to the surface of one- or two-dimensional gels
  2. Direct application of antiserum to a paper transfer of the gel
  3. Passive binding to nitrocellulose sheets
  4. Covalent bonding to diazobenzyloxymethyl paper
  5. Filter affinity transfer
- Identification of surface antigens
  1. Antigen labeling (intrinsic or extrinsic)
  2. Antigen solubilization
  3. Antigen fractionation
  4. Demonstration of cell surface location
- Hybridoma-derived antibodies
  1. Production of monoclonal antibodies
  2. Screening procedures for hybridoma culture supernatants
- Recombinant DNA
  1. Clarification of genomic rearrangements related to antigenic variation
  2. Identification of nucleic acid sequence data
  3. Production of potentially useful parasite polypeptides

It is important to understand the factors influencing the immune responses of the host, including (i) genetics; (ii) state of the host at exposure, including nutrition, age, health status, and underlying diseases; and (iii) the size, route, and frequency of the parasite loading dose. Also, there are several types of resistance, which include natural resistance (how refractory the host is to a particular parasite), acquired resistance (either specific or nonspecific), and immunoregulation (Table 32.3).

Innate Immune System. Anatomical barriers to infections include mechanical factors (epithelial surface physical barrier, desquamation of skin epithelium, cilia movement in air passages, peristalsis in the gastrointestinal tract, flushing of tears, and saliva), chemical factors (fatty acids in sweat, lysozyme and phospholipase in tears, saliva and nasal secretions, low pH of sweat and gastric secretions, defensins, and surfactants), and biological factors (normal flora competitors). However, once infectious agents have penetrated the anatomical barriers, acute inflammation be-

TABLE 32.3 Immunoregulatory systems in the host

Antibody-mediated mechanisms	
	Immunologic blockade
	Polyclonal B-cell activation
	Anti-idiotypic antibodies
Cellular mechanisms	
	Tolerance
	Specific suppressor cells
	Nonspecific suppressor cells

comes a factor. Humoral factors play an important role in inflammation, which is characterized by edema and the recruitment of phagocytic cells (Table 32.4). These humoral factors are found in serum or they are formed at the site of infection.

The innate response is the first to be activated during the response to invading organisms and is due primarily to phagocytic cells. This response is related primarily to immediate recognition and response to pathogen invasion. Phagocytes include polymorphonuclear leukocytes or neutrophils, monocytes, and macrophages (Table 32.4). Secondary phagocytes can include dendritic cells, fibroblasts, and epithelial cells. Macrophages are found in all tissues and represent the first line of defense against pathogenic invasion of the host (Table 32.5). In order to thrive, parasites must be able to survive the innate response. Complement activation represents a first line of defense against extracellular parasites. Once the membrane attack complex is formed, parasite lysis can occur while complement components opsonize parasites for phagocytosis. One of the most important cytokines in cell-mediated innate resistance is interleukin-12 (IL-12). IL-12 activates natural killer (NK) cells, which in turn produce gamma interferon (IFN-γ), which is responsible for activation of effector mechanisms that control replication of intracellular parasites.

Acquired/Adaptive Immune System. Although innate immunity is critical in resistance to acute parasitic infections, the acquired, or adaptive, response is necessary for long-term protective immunity. Adaptive T- and B-cell responses following infection depend on accessory cells that present antigen, provide costimulation, and produce cytokines, all of which impact the onset, duration, strength, and type of the adaptive immune response. CD4$^+$ helper T cells are divided into two subsets, Th1 (type 1 response) and Th2 (type 2 response), each of which produces different cytokines and has different functionalities (Table 32.6). At times, both type 1 and type 2 responses may be used to eliminate the same parasite. These two mechanisms may be functional at the same time, during different stages of the parasite life cycle, or in different locations within the body.

TABLE 32.4 Humoral and cellular barriers to infection (serum or at site of infection)

Immune system factor	Comments
Humoral	
Complement system	Major humoral nonspecific defense mechanism. Once activated, complement can lead to increased vascular permeability, recruitment of phagocytic cells, and lysis.
Coagulation system	Depending on the severity of the tissue injury, the coagulation system may or may not be activated.
Lactoferrin, transferrin	Plasma proteins that bind iron; expressed secretory granules of neutrophils, airway fluid, cervical mucus, breast milk, tears, saliva
Interferons	Proteins that can limit replication
Lysozyme	Breaks down organism membranes; small, abundant cationic enzymes; widely distributed; expressed in PMNs, monocytes, macrophages, keratinocytes, small intestine, tracheal epithelial cells, pneumocytes and apocrine gland secretory cells; found in tears, saliva, breast milk and respiratory fluid
Interleukins	Induce fever, production of acute-phase proteins
Cellular	
Neutrophils	Polymorphonuclear cells (PMNs) phagocytize/kill invaders; contribute to collateral tissue damage during inflammation
Macrophages	Tissue macrophages and monocytes (differentiate into macrophages) function in phagocytosis/intracellular killing; extracellular killing; contribute to tissue repair; act as antigen-presenting cells (required for induction of specific immune responses)
Natural killer (NK) cells, lymphokine activated killer (LAK) cells	Not part of inflammatory response, but important in nonspecific immunity
Eosinophils	Contain protein granules; effective in killing certain parasites

Immunopathologic Reactions in the Host. It is important to have a basic understanding of the types of immunopathologic reactions in the host which are responsible for disease. These are often classified into four categories or pathways by which the host responds. They have been categorized as (i) type I reaction (anaphylactic), initiated by the antigen reacting with tissue cells (basophils and mast cells) passively sensitized by antibody produced elsewhere (release of pharmacologically active substances); (ii) type II reaction (cytotoxic or cell stimulating), initiated by the antibody reacting with an antigenic component of, or antigen associated with, a cell or tissue element (damage occurring in the presence of complement or certain mononuclear cells); (iii) type III

TABLE 32.5 Macrophage functions

Macrophage functions	Mechanisms
Protective	
Detection of pathogen invasion	Opsonic receptors for microbes Complement and antibody receptors Nonopsonic receptors Membrane-bound pattern recognition receptors (C-type lectins, leucine-rich proteins, scavenger receptors, and integrins)
Control of microbial spread	Phagocytosis, granuloma formation, intracellular killing
Recruitment of immune cells	Release of cytokines and other inflammatory mediators
Lymphocyte activation	Antigen presentation, costimulatory molecules, cytokines
Effector cells for cell-mediated immunity	Increased phagocytosis, increased intracellular killing, clearance of apoptotic cells
Participation in humoral immunity	Receptors for antibody and complement
Nonprotective	
Tissue damage	Extracellular release of toxic oxidants, hydrolytic enzymes Inflammatory mediators (TNF-α, IL-1) Fever, wasting, septic shock
Dissemination of pathogens	Transport throughout the host
Autoimmune disease	Presentation of microbial epitopes to lymphocytes, which cross-react with self molecules

TABLE 32.6 Differences between Th1 and Th2 CD4$^+$ T helper cells

Th1: type 1 responses	Th2: type 2 responses
Produce IFN-γ and IL-2; induction of cytolytic activity by CD8$^+$ T cells; production of complement-fixing antibodies	Produce cytokines (IL-4, IL-5, IL-10, and IL-13); associated with high levels of neutralizing and cell-bound antibodies, mast cell activation, eosinophilia, suppression of type 1 responses
IL-12 associated with resistance to protozoa and susceptibility to various helminths	IL-4 plays important role in development of Th2-type responses: associated with resistance to helminths (expulsion of intestinal nematodes) and susceptibility to protozoa
Produce lymphotoxin: promote macrophage activation and generation of cell-mediated immunity	Support maturation of B cells to immunoglobulin-secreting cells (activation of humoral immune mechanisms)
Resistance to intracellular pathogens requires Th1 proinflammatory response	Resistance to extracellular pathogens requires Th2 response
Prevention: IFN-γ, IL-12, IL-10 also cooperate to keep Th2 response in check	Prevention of Th1 response from overproduction: IL-10, TGF -β, IL-4
Problems: effector molecules detrimental if produced too long: nitric oxide, reactive oxygen intermediates (IL-1, IFN-γ, TNF)	Problems: strong antibody responses = antigen/antibody complexes, complement activation, eosinophils = immediate hypersensitivity; can cause hepatic fibrosis, portal hypertension, chronic morbidity

TABLE 32.7 Allergic reactions responsible for disease in parasitic infections[a]

Infection	Clinical disease	Reaction type
Malaria	Nephrotic syndrome	Immune complex (III)
	Anemia	Cytotoxic (II)
Chagas' disease	Myocarditis	Cytotoxic (II)
Leishmaniasis	Spectral disease	Cell mediated (IV)
Schistosomiasis	Swimmer's itch	Anaphylaxis (I)
		Cell mediated (IV)
	Katayama syndrome	Immune complex (III)
	Granulomatous disease	
	Schistosoma mansoni	Cell mediated (IV)
	Schistosoma haematobium	Cell mediated (IV)
Filariasis	Elephantiasis	Cell mediated (IV)
	Tropical pulmonary eosinophilia	Anaphylaxis (I)
Echinococcosis	Leaky hydatid fluid	Anaphylaxis (I)

[a] Data from references 2 and 3.

reaction (antigen-antibody complexes), initiated when the antigen reacts with precipitating antibody (forming microprecipitates in and around small vessels, interfering with membrane function, and forming soluble circulating complexes); and (iv) type IV reaction (delayed, tuberculin type, cell mediated), initiated by reaction of active lymphocytes in response to allergen, release of lymphokines, and/or cytotoxicity (infiltration of cells). These are listed in Table 32.7. The overall immune response to the presence of any parasite is no different from the responses seen with any infectious agent, all of which begin with the organism infecting the host and being recognized by the cells of the immune system. Once the parasite is recognized as foreign by the immune system, a series of steps are initiated (Fig. 32.1; Table 32.8).

Parasites and Avoidance of the Host Immune System. Parasites have three basic characteristics that make it very difficult for the host to control them: parasite size, complicated parasite life cycles, and antigenic complexity. There are also a number of ways in which parasites avoid the host immune system responses: these include (i) location

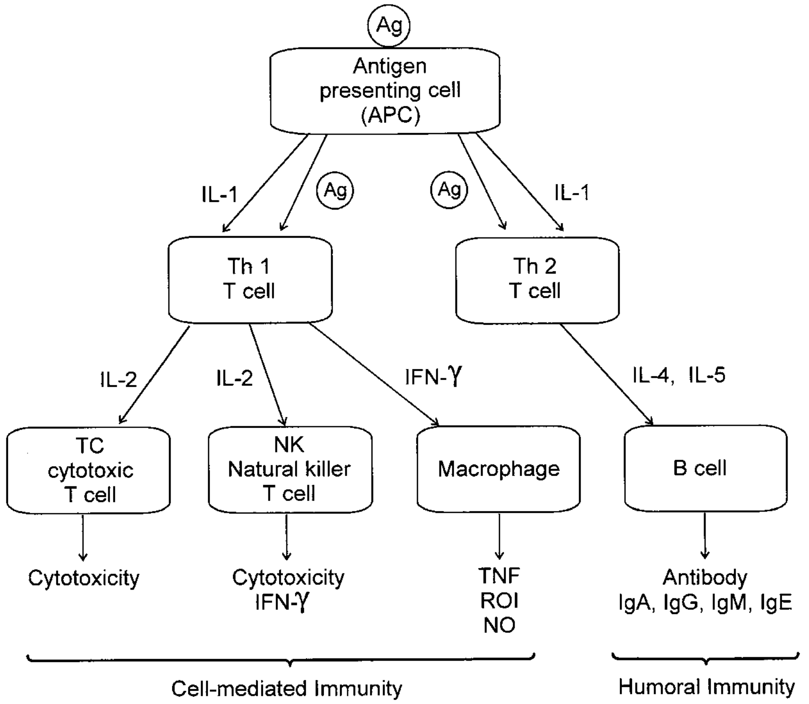


Figure 32.1 Representation of the immune response. Antigen (Ag) is recognized and processed by antigen-presenting cells (APC) and presented to Th1 or Th2 cells that carry receptors for the antigen. At the same time, the APCs release IL-1, a cytokine that activates resting Th1 and Th2 cells. Activated Th1 cells produce IL-2, which activates cytotoxic T (TC) or natural killer (NK) cells. Cytotoxic T cells kill target cells carrying the original antigen in a major histocompatibility complex-restricted way, while NK cells kill target cells nonspecifically. Another product of the Th1 cells is IFN-γ, which activates resting macrophages to become more phagocytic and to release TNF, reactive oxygen intermediates (ROI), and nitric oxide (NO). Both ROI and NO are involved in killing intracellular parasites or those in close proximity to the activated macrophage. The Th2 cells release IL-4 and IL-5, which are involved in B-cell activation and the release of antibodies specific to the antigen. IL-5 also activates eosinophils. Antibodies may cooperate with macrophages and eosinophils to serve as a bridge bringing the activated cell and the parasite together; they may also support the release of toxic molecules directly onto the surface of the parasite. *Note*: It is important to remember that some of the cytokines exert both a positive and negative influence on the immune response at the same time; this diagram is designed to show the main features of the overall immune response. (Adapted from references 2 and 3.) doi:10.1128/9781555819002.ch32.f1

within body sites that are relatively protected from the immune response, (ii) various modifications of surface antigens, and (iii) different mechanisms that can modify the host immune response. A number of these evasion mechanisms are listed in Table 32.9.

All human parasites elicit immune responses, both humoral and cellular. Specific antibodies belonging to the IgM, IgG, IgA, and IgE classes have been found, indicating the body's recognition of parasite antigens (Table 32.10). Cell-mediated immune responses have also been clearly documented. However, in both cases, there may be little or no correlation between responses and protection of the host.

The immune system functions in the same way for parasites as for other organisms; however, there are some dramatic differences based on the complexity of the response. As parasites progress through their normal life cycle stages, many different antigens are expressed at each different stage. This leads to the development of many different antibody-dependent and -independent responses. In some cases, the immune system of the host actually becomes confused, leading to situations where the host is harmed rather than helped.

Although the host response to protozoa is quite different from the host response to helminths, some general statements apply to both situations. When a parasite and the host inter-

TABLE 32.8 Immune system “alphabet soup” and definitions[a]

Term[b]	Definition	Comments
APC	Antigen-presenting cell	Processes the foreign antigen and presents it to the T-helper cells
B cell	Lymphocyte; precursor of plasma cell; expresses immunoglobulins on its surface but does not release them	B lymphocyte; short life; responsible for production of immunoglobulins
T cell	Lymphocyte; helper, killer, suppressor cells	T lymphocyte; long life; responsible for cell-mediated immunity; soluble products are lymphokines
Th	T-helper cell; both Th1 and Th2 carry same $CD4^+$ or $CD8^+$ surface markers	T lymphocyte; Th1 produces IL-2 and IFN-γ; Th2 produces IL-4 and IL-5; may be antagonism between products of Th1 and Th2; Th1 drives immune response toward antibody-independent immune responses; Th2 drives immune response toward antibody production
Lymphokine	Hormone-like peptide, released by activated lymphocytes	Mediates immune responses
Cytokine	Hormone-like low-molecular-weight protein that regulates the intensity and duration of immune responses and is involved in cell-to-cell communication	Interferon (IFN), interleukin (IL), lymphokines; released from spleen, thymus, epithelial, endothelial, and mast cells and by fibroblasts and lymphocytes
Interferon (IFN)	Small glycoproteins; classified into three groups (alpha, beta, and gamma); can regulate many cell properties and functions	IFN-α2A and -2B used as antineoplastic agents; IFN-β elaborated by fibroblasts; IFN-γ elaborated by T lymphocytes in response to specific antigen or mitogenic stimulation
Interleukin (IL)	Name given to cytokines once their amino acid structure is known	IL-1: from mononuclear phagocytes; enhances proliferation of Th and growth and differentiation of B cells IL-2: from Th; causes proliferation of T cells and activated B cells (T-cell growth factor) IL-3: from monocytes, fibroblasts, and endothelial cells; increases the production of monocytes (multi-colony-stimulating factor) IL-4: from T4 lymphocytes; causes differentiation of B cells (B-cell differentiation factor) IL-5: from T cells; causes activation of B cells and influences cytotoxic activity of eosinophils; increase in eosinophils IL-6: from fibroblasts, macrophages, and tumor cells; increases synthesis and secretions of immunoglobulins by B cells (B-cell-stimulating factor 2, IFN-β2) IL-7: from bone marrow cells; causes proliferation of B and T cells IL-8: from endothelial cells, fibroblasts, keratinocytes, macrophages, and monocytes; causes chemotaxis of neutrophils and T cells IL-9: from T cells; causes growth and proliferation of T cells IL-10: from helper T cells, B cells, and monocytes; inhibits IFN-γ secretion by T cells and mononuclear cell inflammation; controls type of immune response IL-11: from bone marrow endothelial cells, macrophages, and preadipocytes; stimulates increased concentrations of acute-phase proteins (C-reactive proteins), mannose-binding protein, serum amyloid-P component, α_{11}-antitrypsin, fibrinogen, ceruloplasmin, and complement components C9 and factor B in plasma IL-12: from B cells, T cells, and macrophages; induces IFN-γ gene expression in T cells and NK cells; controls type of immune response; facilitates formation of Th1 type response IL-13: from Th cells; inhibits mononuclear cell inflammation IL-14: from T cells; stimulates B-cell proliferation and inhibits immunoglobulin secretion IL-15: from T cells; stimulates T-cell proliferation and NK cell activation

(continued)

TABLE 32.8 Immune system "alphabet soup" and definitions[a] *(continued)*

Term[b]	Definition	Comments
NK cell	Natural killer cell; heterogeneous collection of lymphocytes; neither B nor T cells; activated by IL-12	Kills target cells nonspecifically; does not involve MHC[b] class I molecules; is being increasingly implicated in protection against parasitic infections
Cytotoxic T cell	T lymphocyte	Kills target cells carrying original antigen in an MHC-restricted manner; often involved in viral infections but seldom in parasitic infections
TNF	Tumor necrosis factor; a cytolytic factor that is produced by CD4 and CD8 T cells after exposure to antigen; also produced by macrophages	A polypeptide hormone; has the ability to modulate adipocyte metabolism, lyse tumor cells *in vitro*, and induce hemorrhagic necrosis of certain transplantable tumors *in vivo*; TNF-α influences cytotoxic activity of eosinophils
ROI, NO	Reactive oxygen intermediates; nitric oxide	Both are involved in intracellular killing of ingested parasites or destruction of parasites in close proximity to the activated macrophage

[a] Adapted from references 2, 3, 30.
[b] MHC, major histocompatibility complex.

TABLE 32.9 Various methods used by parasites to evade the host immune response

1. Become intracellular (*Plasmodium* spp.)
2. Enter immunologically protected site soon after infection (*Plasmodium* spp.)
3. Leave site of established immune response during life cycle (*Ascaris lumbricoides*)
4. Become disguised with host antigen(s) (*Schistosoma* spp.)
5. Survive in macrophages (*Leishmania* spp., *Trypanosoma cruzi*, *Toxoplasma gondii*)
6. Live in lymphocytes (*Theileria* spp.)
7. Inactivate host lymphocytes (*Schistosoma mansoni*)
8. Cause polyclonal stimulation of lymphocytes (*Trypanosoma brucei* subspp., *Plasmodium falciparum*, *Babesia microti*, *Entamoeba histolytica*, *Trichinella spiralis*)
9. Activate macrophages (*Leishmania* spp., *Toxoplasma gondii*, *Trichinella spiralis*, *Nippostrongylus brasiliensis*)
10. Shed antigens (*Trypanosoma brucei* subspp., *Entamoeba histolytica*, *Ancylostoma caninum*)
11. Undergo antigenic variation (*Plasmodium falciparum*, *Trypanosoma brucei* subspp.)
12. Inhibit cell or antibody binding (*Schistosoma mansoni*)
13. Suppress immune system (*Trypanosoma cruzi*, *Trypanosoma brucei* subspp., *Leishmania donovani*, *Plasmodium* spp.)

act, there are several possible outcomes: (i) the parasite fails to become established in the host; (ii) the parasite becomes established and kills the host; (iii) the parasite becomes established and the host eliminates the infection; (iv) the parasite becomes established and the host, in trying to eliminate the organism, becomes damaged itself; and (v) the parasite becomes established, and the host begins to overcome the infection but is not totally successful (2). Some representative parasites are presented in this chapter. Obviously, some human parasites have been studied more than others, and these differences are reflected in the amount of available information related to parasite immunology.

Amebiasis

Entamoeba histolytica typically establishes a commensal relationship with the host, and cysts of nonpathogenic trophozoites are excreted, thus perpetuating the life cycle of the parasite (Fig. 32.2). It is estimated that 90% of cases are asymptomatic while 10% of infections progress to symptomatic disease. A small but significant subpopulation of symptomatic individuals develops severe extraintestinal amebiasis, including amebic liver abscess. With the development of axenic culture and antigen purification techniques, *E. histolytica* infections and subsequent host immune responses have been more thoroughly investigated. However, although both humoral and cellular responses occur in amebiasis, the role of immunity is still not completely understood.

Pathogenic *E. histolytica* is now well recognized as a separate species from the morphologically identical but

TABLE 32.10 Immune system antibodies (neutralization, agglutination, complement activation, and facilitated opsonization)

Antibody	General comments	Functionality
IgA	Occurs as 2 subclasses (IgA1 and IgA2); can occur in monomeric, dimeric, or polymeric forms; found in secretions, where it protects the mucous surfaces of the gut, respiratory tract, and urogenital tract; occurs in breast milk	Neutralization of bacterial toxins; inhibition of invasion of the mucosa by agglutinating infectious agents or preventing attachment to the host cell
IgG	Found in serum and extravascular spaces; occurs as 4 subclasses, IgG1, IgG2, IgG3, and IgG4	Most common antibody in secondary immune response; all subclasses can agglutinate microorganisms; IgG1, IgG2, and IgG3 activate complement, with IgG3 being the most effective; IgG1 and IgG4 can cross the placenta; IgG1 and IgG3 can bind to macrophages via their Fc portions
IgM	Found in serum and extravascular spaces; pentameric immunoglobulin; single subclass	First antibody produced in response to antigenic stimulation; agglutinates microorganisms with repeated epitopes; activates complement via classic pathway
IgE	Has an affinity for mast cells, basophils, and eosinophils, to which it binds via the Fc portion	When bound to an antigen, it causes cross-linking, resulting in the release of active substances from the cell to which it is bound

nonpathogenic *E. dispar*. *E. histolytica* uses different virulence factors, including a surface membrane GalNAc lectin that allows adherence and induces cytolysis plus a cysteine protease. Cysteine proteases contribute to tissue invasion, causing degradation of the extracellular matrix, and they participate in the induction of apoptosis (programmed cell death) in cells contacted by the amebae (4). They also assist in circumventing the host immune response through cleavage of secretory IgA and IgG and activation of complement (5). Cysteine proteases are encoded by a number of genes present in *E. histolytica* but not *E. dispar*. A detailed study of cysteine proteases may eventually provide the key to the design of specific inhibitors that could serve as new chemotherapeutic agents. Currently, known amebic virulence factors include the Gal/GalNAc adherence lectin, cysteine proteases, arginase, amebapores, alcohol dehydrogenase, peroxiredoxin, cyclooxygenase 2, and lipopeptidophosphoglycan (6–8). Characterized virulence factors of *E. histolytica* are often down-regulated in nonpathogenic isolates or absent in the nonpathogenic amebic species *E. dispar* and *Entamoeba moshkovskii*.

Humoral Immunity

Coproantibodies can be detected in approximately 80% of patients with amebic dysentery, with a steady decline to about 55% over a period of weeks. During the same time frame, a rise in the level of antibodies in serum can be demonstrated. These data may represent a transient secretory response followed by invasion and penetration of the intestinal mucosa with production of circulating antibody. Once invasive amebiasis has been established, a serologic response can generally be demonstrated within a week after the onset of symptoms. These titers tend to be high and can persist for several years, even after invasive disease has healed or a subclinical infection has been controlled. However, this humoral response during the invasive phase of the disease apparently has little impact on potential reinfections or in the healing process.

IgM is produced, although IgG is found at a higher concentration, with the predominant subclass being IgG2 (9). Data indicate that cleavage of IgG by the extracellular cysteine proteinase of *E. histolytica* trophozoites may limit the effectiveness of the host humoral response (10). The production of anti-ameba IgE is controversial, and its role in *E. histolytica* infections is unclear. A cytotoxic effect on the trophozoites is produced, and organisms lose motility when in contact with serum containing specific antibodies. Also, introduction of antibodies to cultures inhibits growth, and antibody-treated organisms do not induce liver abscess in susceptible animal models.

It is also interesting that sera from healthy individuals with no evidence of past or present amebiasis also have the same effects on organisms as immune sera do. Even after absorption with *E. histolytica* trophozoites, the effects persist, suggesting that they are due to causes other than the presence of natural antibodies. Complement activation is involved, via classic and alternative pathways, and apparently does not require antibodies. Activation of complement pathways results in lysis of the trophozoites. It has been reported that highly virulent strains of *E. histolytica* are more resistant to complement-mediated lysis; thus, complement may also play a role in natural resistance to infection by causing a potential reduction in invasive capacity. However, it is not known if reduced complement activity plays a role in the susceptibility of certain individuals to infection or whether it may be related to host defenses.

Although the data are limited, a suppression of humoral response to sheep erythrocytes in hamsters has been documented when extracts of *E. histolytica* were used.

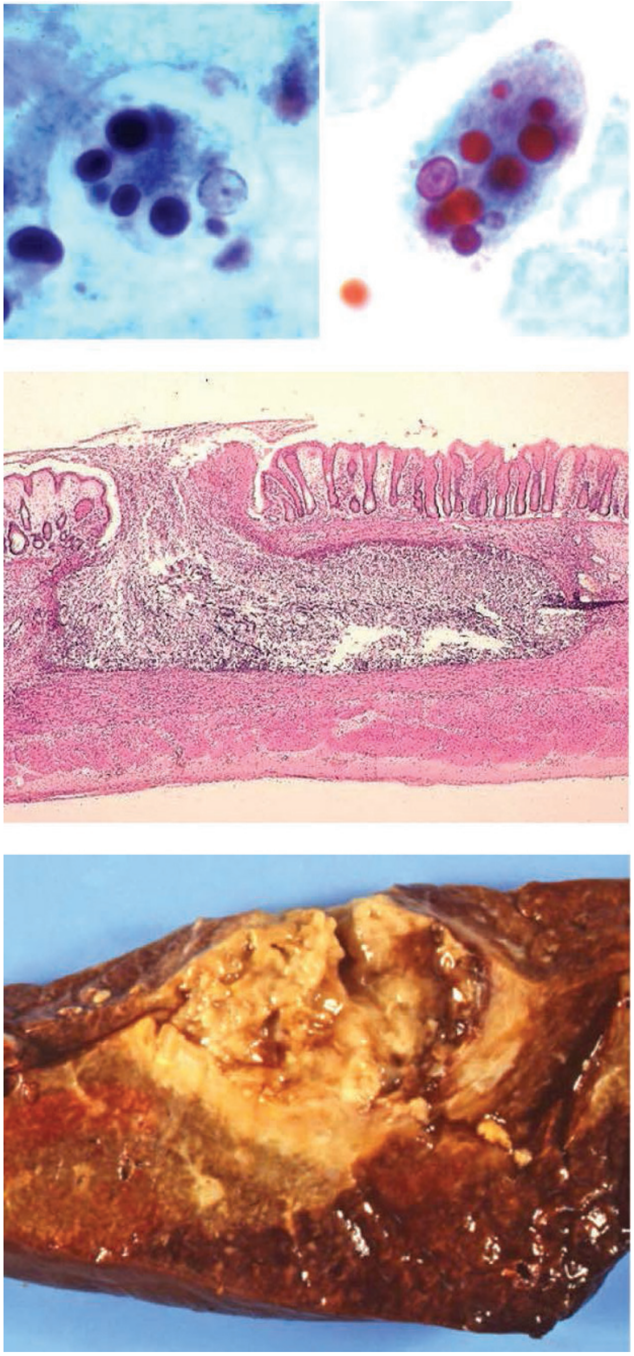


Figure 32.2 *Entamoeba histolytica*, amebiasis. (Top) Two examples of trophozoites containing ingested RBCs within the cytoplasm (morphologic evidence of true pathogen, *E. histolytica*). (Middle) Amebic intestinal "flask-shaped" ulcer. (Bottom) Amebic liver abscess (Armed Forces Institute of Pathology photograph). doi:10.1128/9781555819002.ch32.f2

The effect is enhanced when the erythrocytes and amebic extract are injected on the same day; however, the primary response only is involved. Suppression of the primary response has also been documented with ovalbumin as the antigen.

Survival mechanisms allow the survival of the parasite, often in spite of both natural and immune defenses. One of these mechanisms is capping, i.e., surface redistribution of membrane antigens in amebae, which may circumvent the effect of surface-bound specific antibodies. Antibodies to the amebae bind to the organism and induce a polar redistribution of surface components. The surface becomes an accumulation of folded membranes that extrude as a cap, containing most of the antibodies that were originally bound to the surface. The cap is then released, probably by constriction of a limiting membrane region.

In spite of the production of high levels of antibody in response to an infection with *E. histolytica*, it appears that the humoral response is not protective to the host. This is confirmed by the ease with which reinfection occurs and the fact that high antibody levels can be seen in patients who remain symptomatic with amebiasis.

The identification and structural characterization of three amebic antigens, the serine-rich *E. histolytica* protein (SREHP), the 170-kDa subunit of the Gal/GalNAc-binding lectin, and the 29-kDa cysteine-rich protein, indicate that they all show promise as recombinant antigen-based vaccines to prevent amebiasis (11–14). Recent data confirm that DNA vaccination with SREHP can provide high levels of protection against amebic liver abscess in animal models of disease (15).

Secreted immunoglobulins, defensins, and cathalecidins reinforce mucosal barrier function, actively preventing pathogenic colonization by ameba.

Cellular Immunity

Most research on cell-mediated immunity in amebiasis has been conducted with animal models of amebic liver abscess. However, a number of different approaches to clarify the role of cellular immunity in humans have been taken. One example involved the use of skin test antigens obtained from *E. histolytica* culture. This procedure detects amebiasis long after the patient has recovered from the infection, and it has been useful in epidemiologic studies; patients with invasive amebiasis exhibit a typical delayed-type hypersensitivity reaction, although the reaction is usually negative during the acute phase of an untreated liver abscess (9).

Trophozoites that breach the epithelial barrier elicit a rapid infiltration of neutrophils to the site of infection in early stages of intestinal disease. Intestinal epithelial cells secrete IL-8, a potent neutrophil chemoattractant, when exposed to *E. histolytica*. It is generally accepted that neutrophils are responsible for much of the tissue damage associated with amebic colitis.

Macrophages play important roles in the pathogenesis of both intestinal and hepatic amebiasis. Macrophages are activated by *E. histolytica* trophozoites and are amebicidal when stimulated with IFN-γ, and tumor necrosis factor alpha (TNF-α). The amebic surface component lipopeptidophosphoglycan activates an inflammatory macrophage response upon toll-like receptor 2/6 (TLR2/6) and TLR4 binding. *E. histolytica* DNA also promotes macrophage activation by TLR9 binding. Furthermore, macrophages upregulate TLR2 in response to the amebic galactose-specific lectin. Macrophage amebicidal activity is mediated by nitric oxide (NO) synthase. NO inhibits important amebic virulence factors including the cysteine proteases and alcohol dehydrogenase 2.

Macrophage migration-inhibition assays have shown results similar to those obtained with skin test antigens during invasive disease. Results were negative during the early phases of liver abscesses but became positive after therapy. A number of different animal model studies also confirm that cell-mediated immunity plays an active role in the overall immune response to infection with *E. histolytica* (9).

Concanavalin A-induced lymphocyte transformation with cells from both patients with amebic liver abscess and healthy controls is comparable; similar results have also been demonstrated with phytohemagglutinin. However, studies with infected hamsters indicated reduced reactivity to concanavalin A and phytohemagglutinin; other reports indicate that *E. histolytica* extracts are mitogenic for murine lymphocytes. Induced blastogenesis in lymphocytes from patients with amebic liver abscess has been shown with aqueous *E. histolytica* extracts and a subcellular antigenic fraction, thus indicating *in vivo* sensitization of T lymphocytes to amebic antigens (9).

According to Ortiz-Ortiz (9), all arguments in favor of protective immunity in amebiasis are based on cellular immunity, hence the emphasis on studies involving the *in vitro* interaction of *E. histolytica*, T lymphocytes, and macrophages. A compilation of some of these studies can be seen in Tables 32.11 and 32.12. It is apparent from these studies that cell-mediated immunity limits the clinical manifestations of amebic liver abscess and protects against recurrent infections.

Most studies reinforce the idea that cell-mediated immunity is the most important factor in acquired protective immunity. A number of factors support this conclusion: (i) cellular anergy that supports the initial *E. histolytica* invasion; (ii) increased incidence of invasive amebiasis after T-cell suppression or splenectomy; (iii) protective impact of T-cell stimulants; (iv) after recovery from amebic liver abscess, appearance and persistence of a delayed-type hypersensitivity reaction to amebic antigens; (v) transfer to immunity via sensitized T cells; and (vi) the lysis effect of cytotoxic T cells stimulated with antigen and activated macrophages.

TABLE 32.11 Interaction between *Entamoeba histolytica* and T lymphocytes *in vitro*

Study	Comments
Virulent *E. histolytica* with lymphocytes from patients who have recovered from amebic liver abscess	Parasite lysis (also with lymphocyte supernatant fluids)
E. histolytica with lymphocytes, peritoneal cells, or spleen cells from infected hamsters	Parasite lysis; may have been caused by lymphokine-activated macrophages
Lymphocytes (treated amebic liver abscess patients) with ameba antigen or nonimmune T cells (PHA[a] stimulated)	Parasites killed
E. histolytica with supernatant fluids of stimulated T cells	Inhibition of DNA and protein synthesis; not toxic

[a] PHA, phytohemagglutinin.

Anergy during the course of amebic liver abscess is well documented. Animal models reveal tissue destruction with minimal inflammatory reaction surrounding these abscesses. One explanation for this anergy might be a temporary desensitization caused by circulating antigen; it is not thought to be due to decreased T-cell numbers, since normal levels have been detected in patients with amebic liver abscesses (9).

Phagocytosis by both peripheral blood polymorphonuclear leukocytes and monocytes is reduced in various circumstances related to amebiasis. Phagocytic function in polymorphonuclear leukocytes was more depressed in patients with amebic liver abscess than in patients with other manifestations of *E. histolytica* infection or in healthy controls. In evaluating the functional capacity of monocytes, phagocytosis was significantly depressed in both liver and intestinal amebiasis and bactericidal function was depressed even more in liver amebiasis.

Studies of patients with amebic liver abscess that had been healed for a minimum of 9 weeks indicated that the $CD4^+$ (helper, inducer T-cell) lymphocyte subpopulation was smaller and the $CD8^+$ (suppressor, cytotoxic T-cell) subpopulation was increased, resulting in a lower cell ratio in patients with amebic liver abscess than in controls (16). However, when the same group of patients was checked a year later, four of five demonstrated an increased $CD4^+$ cell percentage and three of five showed an increased ratio of $CD4^+$ to $CD8^+$ cells. Since T lymphocytes from patients cured of amebic liver abscesses have been shown to destroy *E. histolytica* after activation with parasite antigens, this may have an impact on host resistance in these patients.

TABLE 32.12 Interaction between *Entamoeba histolytica* and macrophages *in vitro* and *in vivo*

Study[a]	Comments	Reference(s)
Virulent *E. histolytica* with nonactivated macrophages	Lysis of mammalian cells; no harm to amebae	95
Virulent *E. histolytica* with activated macrophages (lymphokines induced with ameba soluble protein, ConA, or PHA)	Amebae killed; dependent on cell-cell contact, time, and oxidative and nonoxidative processes; decrease in macrophage viability, possibly owing to lysis by *E. histolytica* toxic products	96
Silica poisoning of macrophages or use of antimacrophage serum	Increased susceptibility to amebic liver abscess; larger abscesses; dissemination to other tissues (hamsters, guinea pigs)	96, 97
Bacillus Calmette-Guérin-stimulated macrophages	Decreased susceptibility to amebic liver abscess; smaller abscesses; less dissemination (to hamsters, guinea pigs)	96, 97
Congenitally athymic nude mice (*nu/nu*), macrophage dependent	Resistant to invasive amebiasis; can limit development of liver abscess	98, 99

[a] ConA, concanavalin A; PHA, phytohemagglutinin.

Natural Immunity in Humans

Certain HLA class II alleles and haplotypes are associated with drastically decreased rates of *E. histolytica* infection (8). Conversely, a single amino acid replacement (Q223R) in the extracellular domain of the leptin receptor encoded by a single nucleotide polymorphism is associated with a nearly 4-fold increase in susceptibility to infection by *E. histolytica* as well as an overall decrease in time to infection (8). It is clear that the host genetic background influences susceptibility to amebiasis. Hopefully, the identification of additional host genetic factors will lead to a better understanding of the pathogenesis of amebiasis, along with enhanced therapeutics in these specific populations.

Giardiasis

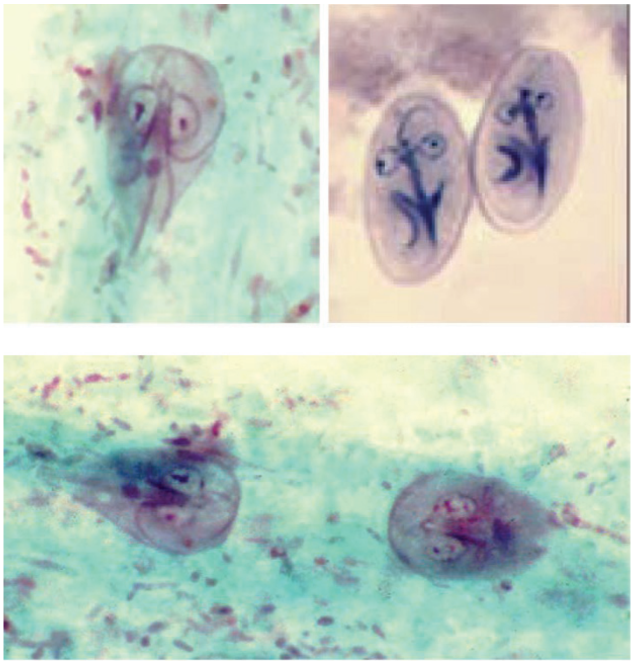
Giardia lamblia is one of the most commonly diagnosed flagellates in the intestinal tract and in many areas of the world (Fig. 32.3). Although various criteria have been used to differentiate species of *Giardia*, there is still disagreement about classification and nomenclature. On the basis of different species names for different animal hosts, there are approximately 50 *Giardia* species. However, there are also data to suggest either biological or antigenic differences among many of these species.

Studies during the last 15 years have confirmed that *Giardia* organisms elicit an immune response that probably plays a role in the variable host response generated to the presence of the parasite. Early data indicating a lower incidence of symptoms in individuals who were repeatedly exposed to the organism suggested the presence of an immune response. Subsequent studies with humans and experimental animals have confirmed the presence of both humoral and cellular immune responses to *Giardia* spp.

In giardiasis a diffuse loss of microvillous brush border, usually combined with villous atrophy, is responsible for malabsorption, which ultimately causes diarrhea. Other mucosal changes include crypt hyperplasia and increased infiltration of intraepithelial lymphocytes. The loss of brush border surface area, reduced disaccharidase activities, and increased crypt/villus ratios are mediated by CD8$^+$ T cells, while both CD8$^+$ and CD4$^+$ small mesenteric lymph node T cells regulate the influx of intraepithelial lymphocytes (17).

Antigenic variation has been documented in giardiasis, and variation at the surface membrane of trophozoites occurs frequently. These antigens are composed of cysteine-rich proteins, and B-cell-dependent mechanisms are probably responsible for the surface antigen switch (18). Apparently, individual organisms express only one variant-specific pro-

Figure 32.3 *Giardia lamblia*, giardiasis. (Upper, left) *Giardia* trophozoite; (right) *Giardia* cysts. (Lower) *Giardia* trophozoites within mucus (fecal specimen).
doi:10.1128/9781555819002.ch32.f3



tein at a time; the detection of multiple surface-labeled bands in some of the studies resulted from subpopulations of trophozoites expressing several different variant surface protein types (19).

Humoral Immunity

The majority of infected patients produce detectable levels of *Giardia*-specific antibodies; however, the biological role played by these antibodies in the host immune response to the infection is unclear. Diagnostic procedures have been developed for the detection of short-lived IgM anti-*Giardia* antibodies and IgG antibodies that may remain at high titers for many months after the patient has been treated and cured. Specific anti-*Giardia* IgM responses in serum were found in 100% of human volunteers experimentally infected with human-source *Giardia* spp. (20). Some studies indicate a correlation between the presence of antibody and symptoms in the patient. Other studies, using different antigens and antibody detection procedures, fail to confirm this correlation.

In asymptomatic patients, the presence of antibody may play a protective role or serve merely as an indicator of infection. Certainly, data indicate that people who are repeatedly exposed to the parasite will tend to be asymptomatic and will have high levels of *Giardia*-specific antibody in serum and breast milk. Patients with hypogammaglobulinemia also have a high rate of diarrhea, often due to *Giardia* spp., and children with chronic diarrhea and giardiasis have an increased incidence of hypogammaglobulinemia. Also, AIDS patients who have giardiasis but no specific antibody also have acute symptomatic infections (21). In contrast to this group of AIDS patients, those who have giardiasis with circulating *Giardia*-specific antibody may have symptoms unrelated to their *Giardia* infection.

Giardia-specific IgA may also be important in both defense against and clearance of the parasite. In one study, patients with symptomatic giardiasis had higher levels of IgA in serum than did uninfected controls and none of the patients were IgA deficient (21). The degree of protection of breast-fed infants against *Giardia* infection also depends on the level of IgA-specific antibodies in breast milk. In the intestine, IgA antibodies may influence the local immune response by inhibiting parasite adherence, tissue invasion, or penetration of the mucosa by parasite antigens (21). IgA anti-*Giardia* antibodies are the most common immunoglobulin type found in acutely ill patients with giardiasis, and they have been detected in persons exposed to a waterborne outbreak in Vermont and villagers in Thailand (21). The jejunal-plasma immune response to *Giardia* involves a decrease in the number of IgA cells and an increase in the number of IgM cells. The large population of antibody-producing plasma cells that are secreting mainly IgA is evidence of the importance of mucosa-associated lymphoid tissue in giardiasis.

Antigenic variation, a mechanism known for many pathogens to evade the humoral immune response of their vertebrate hosts, was shown to occur on the membrane surface of *Giardia* trophozoites both *in vitro* and *in vivo*. Individual *Giardia* trophozoites express only a single species of variant-specific surface protein (VSP) on the surface of the cell membrane at any given time and are subject to replacement by another VSP every 6 to 13 generations of cell growth (22). Using newly established criteria, the total number of recognized VSPs in *Giardia* trophozoites is now refined to 73 (formerly six experimentally identified).

Another protozoan parasite known to possess a single species of variant surface glycoprotein (VSG) covering the cell membrane surface is the African trypanosome *Trypanosoma brucei*. The bloodstream form of the *T. brucei* cell expresses a single species of VSG on the membrane surface at any given time, but this is regularly replaced by another VSG with an estimated frequency of up to 10^{-3} per cell per generation in an apparent effort toward evading the host immune response.

Cellular Immunity

Antibodies to *Giardia* spp. work together with cellular immune mechanisms. Cellular responses to giardiasis occur at the level of the intestinal mucosa, and a number of inflammatory changes may occur during the infection. Histologic changes during giardiasis include infiltration of polymorphonuclear leukocytes into the epithelium and mononuclear leukocytes into the lamina propria, development of shortened villi, loss of the brush border, damage to epithelial cells, and an increase in epithelial cell mitosis (21). In rare cases, giardiasis is associated with changes that mimic celiac sprue and include complete villous atrophy, dense mononuclear cell infiltration, and flattening of the epithelial cells. This accumulation of inflammatory cells in the intestinal mucosal cells of patients with giardiasis suggests that cellular responses play a role in the overall immune response to *Giardia* infection. However, while these histologic findings suggest a cellular immune response, they may also represent a nonspecific inflammatory response stimulated by the presence of an intraluminal infection.

Experimental animal studies provide evidence that T lymphocytes and Peyer's patch helper T lymphocytes play a role in the host immune response. In athymic mice, which are deficient in both T lymphocytes and Peyer's patch helper T lymphocytes, inoculation with *Giardia muris* results in a chronic infection with large numbers of organisms. In contrast, immunocompetent mice clear the parasite and may develop resistance to reinfection.

Several effector mechanisms have been proposed to eradicate *Giardia* infections, including phagocytosis by macrophages; secretion of defensins, NO, and mucins by epithelial cells; and the recruitment and activation of mucosal mast cells (23). In one study, mast cell counts correlated with epithelial permeability and antigen uptake, suggesting a link between development of other immune responses and mast cell recruitment. Reports also indicate that human mononuclear phagocytes are capable of killing *Giardia* organisms and phagocytizing *Giardia* trophozoites (21). Also, gut-associated lymphoid tissue provides the precursor cells that migrate to the mammary gland and become cells capable of secreting the immunoglobulin components of milk. Despite the number of studies on the immunology of giardiasis, the actual effect of antibody or cellular components on the organism itself is unclear. Future studies on defining specific antigens to which the host immune system responds will play a major role in our understanding of this very common infection. For reviews of current information, see references 18 and 21.

Giardia-specific cellular and humoral immune responses have been studied in mouse models. In mice, αβ T-cell receptor-expressing T cells are required to control infection, and diminished CD4 T cells result in chronic infection. Neither a T-helper 1 (Th1) response nor a Th2 response is absolutely required for protection against *Giardia* infection. CD8 T cells seem not to be important for the control of infection in mice but contribute to giardiasis-related intestinal mucosal injury. Important roles have also been shown for mast cells and interleukin (IL) 6, as well as for B-cell antibody production. Human intestinal lymphocytes stimulated by *Giardia* trophozoites demonstrate the importance of IFN-γ, probably secreted by CD4 T cells (24). In summary, data suggest that *Giardia* actively interferes with host innate immunity, resulting in an immune response able to control the infection but lacking strong inflammatory signals.

Toxoplasmosis

Toxoplasma gondii is an obligate intracellular parasitic protozoan that has a wide host range and causes a zoonotic parasitosis called toxoplasmosis (Fig. 32.4). This infection causes significant morbidity. The most effective measures to minimize disease sequelae are prompt diagnosis and treatment and preventing infection. Natural infection usually occurs from direct contact with oocysts or by ingesting undercooked meat containing bradyzoite cysts. The gastrointestinal route appears to be the most common site of infection with *T. gondii*, and, after disruption of the host cells, the organisms invade adjacent cells and are spread throughout the body via the lymphatics and bloodstream. Since the advent of AIDS, the impact of toxoplasmosis as a major opportunistic infection has led to extensive research in the field of immunology. Infection with *T. gondii* stimulates the development of both humoral and cellular immune responses, with the cell-mediated response being the more predominant. Control of *T. gondii* requires the interaction of multiple soluble (i.e., IL-12, IFN-γ) and cellular components (NK cells, dendritic cells, macrophages, and CD4$^+$ and CD8$^+$ T cells) of the host immune system. These immune responses of the host can dramatically affect the course and severity of the infection.

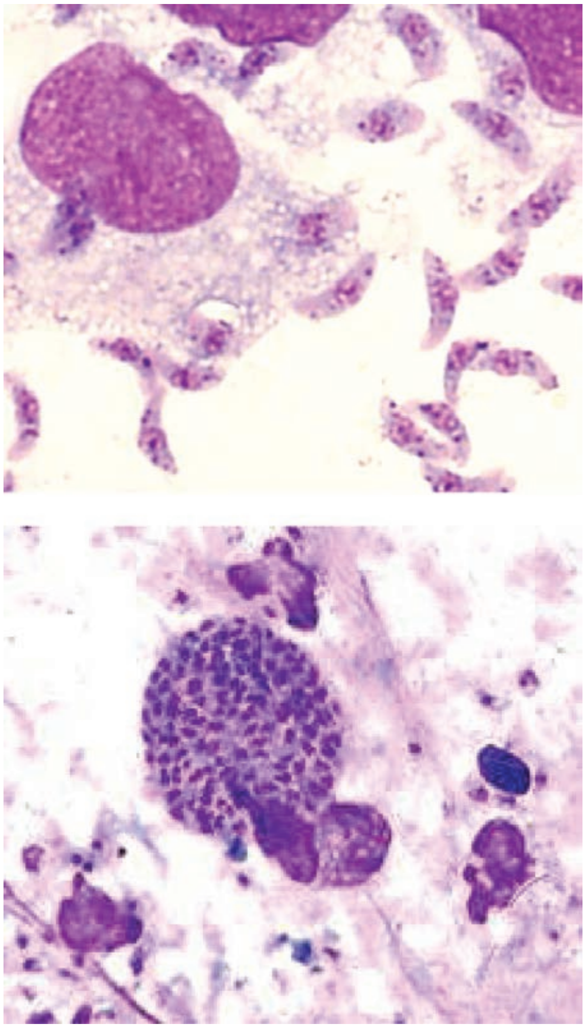


Figure 32.4 *Toxoplasma gondii*, toxoplasmosis. (Upper) *Toxoplasma* tachyzoites (actively proliferating form). (Lower) *Toxoplasma* bradyzoites (resting forms).
doi:10.1128/9781555819002.ch32.f4

Adhesion to the host cell is facilitated by secreted proteins, and invasion uses an actin-myosin-based process. Within the cell, the parasite remains inside the parasitophorous vacuole; the organism prevents the cell from fusing with lysosomes. Eventually the tachyzoites transform into bradyzoites and encyst. Ongoing encystment depends on an ongoing type 1-mediated immune response; IFN-γ and CD4$^+$ and CD8$^+$ cells play a protective role. Although this immune response limits the tissue invasion of the parasite, it also aids the survival of *Toxoplasma* by converting it into a bradyzoite, the resistant form (25).

Humoral Immunity

In spite of the very high antibody titers to *T. gondii* often seen in human infections, humoral antibody plays a secondary role in contributing to protective immunity. The classic approach to the diagnosis of toxoplasmosis has always been based on the detection of immunoglobulins, specifically IgM and IgG. In infected humans, IgM usually appears within 2 weeks after infection, whereas IgG antibodies appear later and their levels peak at approximately 2 months postinfection. The IgG titer decreases gradually to a low level and remains at this level thereafter. For this reason, the presence of IgG demonstrates proof of a prior infection with *T. gondii.* Although detection of IgM has always been considered an indicator of acute infection, the development of more sensitive diagnostic methods means that IgM can now be detected months to years after the initial acute infection. Unfortunately, these findings also complicate the interpretation of serologic test results in patients with toxoplasmosis.

IgA antibodies can be detected early in infection, and their level decreases between 3 and 9 months after infection. The detection of both IgA and IgM at the same time indicates the acute phase of toxoplasmosis and is currently used for risk evaluation of pregnant women. Apparently, IgE antibodies also appear early in infection and then their level begins to decline; the detection of IgE could also serve as an indicator for acute disease. One study indicated the presence of IgE in 86% of infected patients (26). The presence of IgE in immunosuppressed individuals appears to be correlated with the onset of complications such as adenopathies, chorioretinitis, and disease reactivation (27).

Unfortunately, in spite of what is currently known about the humoral response in toxoplasmosis, routine serologic tests are often inadequate for the diagnosis of congenital disease. Specific IgG can persist in a child for months and cannot be differentiated from maternal IgG. However, both IgM and IgA antibodies do not cross the placenta and can be detected in infected fetuses as early as the second trimester of pregnancy, assuming that the mother became infected soon after conception (28). Since children at 1 year of age have only 20% of the level of IgA in adults, early synthesis of specific IgA might also be an indicator of congenital infection (29).

A number of attempts have been made to confer resistance to *T. gondii* by passive transfer of immune homologous or heterologous antisera. The results of these studies have indicated conflicting outcomes; some investigators have reported no protection, whereas others have reported significant protection. The results appear to be related to the choice of experimental host species, specific parasite strains, and transfer of small volumes of high-titer antisera. It appears that anti-*Toxoplasma* antibody can be protective under carefully controlled conditions.

When extracellular tachyzoites are exposed to antibody in the presence of complement *in vitro*, their morphology is altered: the cells become vacuolated and coarse, collapse, and lyse. The changes in dye permeability in the Sabin-Feldman dye test are based on these changes seen with a combination of antibody and complement. However, the *in vivo* intracellular location of the parasite protects it from the circulating antibody.

T. gondii patients have a 2-fold higher level of IL-6 as compared to healthy subjects, which seems to confirm the presence of an inflammatory state. The major function of IL-6 is through the action on B lymphocytes. It is a mediator responsible for the production of acute phase proteins and increased cytotoxic activity of NK cells. IL-6 is an early and sensitive, although nonspecific, marker of inflammatory states.

Also, IL-10 is five times higher in patients with toxoplasmosis than in healthy controls. IL-10 plays an essential role in the inflammatory response during acute *T. gondii* infection, since it inhibits the cellular-type immune response (IL-12, TNF-α) and inflammatory response (IL-6). IL-10 counteracts the harmful effects of the inflammatory response, which is based on the increased production of TNF-α, IFN-γ, and NO associated with intestinal multiplication of *T. gondii*. IL-10 deactivates macrophages, induces γ and facilitates intracellular parasite survival. IL-10 induces immunosuppression during *T. gondii* invasion, which is beneficial both for the host and the parasite (30).

Cellular Immunity

Cellular immunity is the key component of the host's immune reaction to toxoplasmosis. Macrophages, T lymphocytes, and NK cells, as well as the cytokines, are major components of the immune response (21). A number of studies have shown that there is a lower level of lymphocyte transformation in patients with acute disease than in chronically infected patients. In the presence of parasite antigens, lymphocytes from patients with evidence of infection for more than 1 year proliferate, whereas lymphocytes from seronegative patients do not (21).

Animal studies have confirmed the key role of T lymphocytes in protective immunity against *T. gondii.* In infected mice, depletion of CD4$^+$ T lymphocytes reactivates toxoplasmosis in the central nervous system. These cells also play a role in the development of host resistance and in controlling brain cyst development. Apparently, in the mouse model there is a synergistic relationship between

$CD4^+$ and $CD8^+$ T-cell subsets, with $CD4^+$ cells participating in the development of resistance during vaccination or acute infection and $CD8^+$ cells being involved in the maintenance of a protective immune response and the inhibition of cyst formation. $CD4^+$ and $CD8^+$ cells are the key players involved in host resistance in toxoplasmosis. The $CD8^+$ cells are activated by IL-2 secreted by the $CD4^+$ cells, and they exhibit cytotoxic activity against tachyzoites or cells infected with *T. gondii.*

Studies of patients with acute toxoplasmosis have shown a significant elevation in levels of suppressor T cells in patients with symptoms lasting more than 4 weeks. A reversed $CD4^+/CD8^+$ cell ratio is also seen and persists for 2 to 4 months after infection. By using flow cytometry and differential leukocyte counts, an increase in the absolute number of $CD8^+$ T cells was confirmed in patients with symptomatic toxoplasmosis, with no significant change in $CD4^+$ cell levels. Also, cloned $CD4^+$ and $CD8^+$ cells from a symptomatic patient produced less IFN-γ after stimulation with *T. gondii* antigen than did T cells from an asymptomatic patient.

Immunosuppression

Although information continues to expand, it is known that $CD8^+$ cells can be stimulated by *T. gondii* antigen to suppress lymphocyte proliferation; both the suppression and increase in the level of the $CD8^+$ T-cell subset correlate with a more severe and prolonged clinical course (31). This study also supports the suggestion that suppressor T cells play a role in prolonging the symptoms of toxoplasmosis. Lymphadenopathy and elevated numbers of $CD8^+$ T cells have been found in patients with lymphatic toxoplasmosis; the same picture is also seen in patients with viral infections known to cause mononucleosis (Epstein-Barr virus and cytomegalovirus). Overall, the immunosuppressive effect seen in toxoplasmosis is apparently limited to the T-cell responses, while B-cell functions are unaffected.

In congenital infections, T-cell functions are also impaired; studies indicate that most congenitally infected infants demonstrate specific immunologic tolerance to *T. gondii* (32). The presence of the parasite at a critical stage of fetal development results in production of lymphocytes that are unable to distinguish immunologically between parasite and self. It is possible that increased numbers of suppressor T cells that occur during fetal life could induce tolerance by the inhibition of other lymphocytes and production of less IFN-γ and IL-2. Also, in symptomatic babies, these diminished responses to *T. gondii* antigens usually correlate with more severe disease.

In normal, unstimulated macrophages, *T. gondii* tachyzoites can continue to multiply; however, in activated macrophages, vacuoles containing living tachyzoites fuse with lysosomes and the parasites are killed. Both oxygen-dependent and oxygen-independent mechanisms are involved in parasite killing.

There is also evidence to indicate that platelets are cytotoxic for tachyzoites when direct cell-to-cell contact is established, even in the absence of antibody. These findings certainly suggest a role for platelets as one of the mechanisms in host defense against toxoplasmosis.

In two studies, a protective effect of vaccination of mice with a combination of antigen and IL-12 or IL-15 has been demonstrated. In the study using a recombinant surface antigen (rSAG1) plus IL-12, this combination reduced the parasite load in the brain by 40% (33). The second study indicated that the γδ T-cell response to *T. gondii* was enhanced by the administration of exogenous IL-15 in the presence of parasite lysate antigen; the survival times after lethal challenge were lengthened (34). In this study, IgG1 and IgG2a antibody titers were increased, as was splenocyte lymphoproliferation.

African Trypanosomiasis

Human african trypanosomiasis, or sleeping sickness, is a neglected tropical disease of significant public health importance across much of Africa; nagana is the corresponding disease in livestock (94). According to recent data, 50 million people are at risk of contracting trypanosomiasis. In Africa, the extensive distribution of the tsetse fly vector, *Glossinia* sp., has rendered ca. 10^7 km^2 of potential grazing land unavailable for livestock; approximately one-third of the cattle are at risk of trypanosomiasis. Trypanosomiasis manifests in humans as either the acute form, caused by *Trypanosoma brucei rhodesiense*, or the chronic form due to *T. b. gambiense* (Fig. 32.5). In either case, the trypanosomes live in the bloodstream and are directly affected by the production of antibodies by the host.

Humoral Immunity

In reviewing the immune system response of the host to the presence of these parasites, it is important to understand the concept of antigenic variation. It has been long recognized that African sleeping sickness is characterized by a series of parasitemia peaks in the blood. This successive wavelike pattern is due to the appearance of trypanosomes expressing a different antigenic identity. Organisms that have changed their antigenic identity can escape the host immune response and can initiate another increase in the level of parasitemia. The success of this infection depends on the parasite's ability to select between hundreds of genes that encode the variant surface glycoprotein (VSG) or variant surface protein (VSP). Both designations are correct. As the host immune response produces high levels of antibody against one variant, parasites expressing a different VSG gene begin to proliferate. These organisms

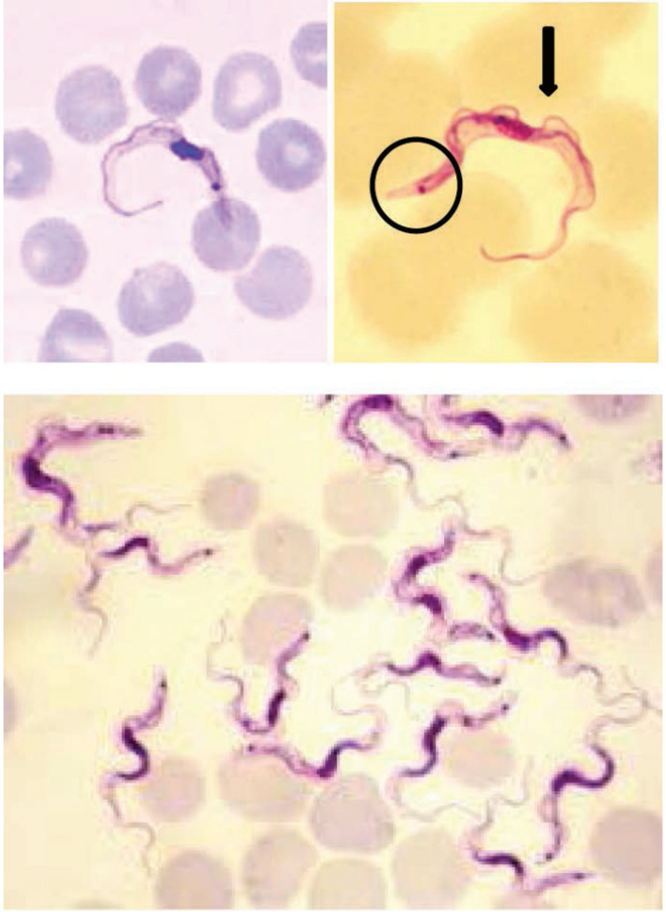


Figure 32.5 *Trypanosoma* sp., African trypanosomiasis. (Upper) *Trypanosoma brucei gambiense*/*T. b. rhodesiense* trypomastigotes in thin blood films; note the very small kinetoplast (circle) and undulating membrane (arrow). (Lower) African trypanosomiasis, heavy parasitemia. doi:10.1128/9781555819002.ch32.f5

persist until they are killed by the immune response, and this pattern is repeated multiple times (35).

We now understand VSG to be a 58-kDa glycophosphatidylinositol-anchored glycoprotein, having predominantly an α-helical secondary structure, with highly variable sequences embedded at the N terminus and monoallelically expressed from a repertoire of hundreds of genes. The factors that induce VSG switching have not been totally defined; however, the presence of host antibodies is not necessary for the changes to occur. The number of variable antigenic types that can occur in any one trypanosome population is quite large (100 or more), and more than 1,000 VSG genes have been detected. There is evidence to indicate that the VSG-specific antibodies represent a mixture of T-dependent and T-independent antibodies directed at different epitopes (36). Unfortunately, although these antibody responses play a role in the successive wavelike growth patterns of the organisms, they do not generate long-lasting immunity.

To be expressed, a particular VSG gene must be located in one of 15 telomeric regions termed "VSG expression sites" (ESs), each of which contains a polycistronic transcription unit that includes ES-associated genes. Only one ES is fully active at a time, so only one VSG gene is transcribed per cell. Although this monoallelic expression is controlled at the transcriptional level, the precise molecular mechanism for this control is not understood (37).

Specific changes in the humoral component of the immune response to trypanosomes include an increase in the cellularity of the spleen and lymph nodes, an increase in the proliferation of spleen cells, an increase in the number of B cells (not T cells), an increase in the IgM level, the appearance of antibodies reacting with nontrypanosome antigens, and the appearance of autoantibodies, rheumatoid factors, and immune complexes (38).

Cellular Immunity

Studies of TNF-α have shown that it is involved in many physiologic functions, and it has been implicated in the pathogenesis of septic shock and systemic inflammatory reactions. Increased levels have been seen in sera from patients with other parasitic infections such as malaria and leishmaniasis; in patients with malaria, increased levels of TNF-α correlate with disease severity. In a recent study, an increase in the level of TNF-α was observed in sera from patients with African trypanosomiasis compared with controls. Patients were classified as being in the early stage (blood, lymphatic) or late stage (meningoencephalitocytic) of the disease. An increase in the level of TNF-α was also noted in late-stage patients compared with those in the early stages of the disease. Data indicated that levels of TNF-α in the serum of patients with African trypanosomiasis were definitely correlated with disease severity (signs of inflammation or presence of major neurologic signs), indicating that TNF-α could be involved in at least some aspects of physiopathology in human African trypanosomiasis (*T. brucei gambiense*) (39).

Immunosuppression

It is also well documented that another key feature of African trypanosomiasis is suppression of the host immune system. Increased incidence of opportunistic infections in patients with African trypanosomiasis is well recognized and has been known since the early 1900s. Both T-cell and B-cell functions can be severely suppressed.

Animal studies have demonstrated two different pathways, one that is suppressor cell dependent and one that is suppressor cell independent. Consequently, suppression has been attributed to polyclonal activation of B cells and to the generation of suppressive cells, two different pathways that are probably coexisting mechanisms.

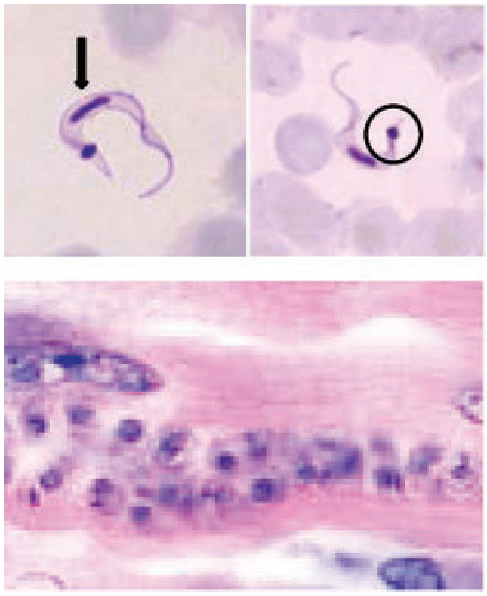
Prostaglandins also play a large role, not only in immunosuppression but also in other areas such as immune system modulation and pathogenesis. It has been shown that prostaglandins in cerebrospinal fluid from patients with sleeping sickness are probably responsible for sleep induction (40).

American Trypanosomiasis

American trypanosomiasis (Chagas' disease) is caused by *Trypanosoma cruzi* and is one of the major health problems in Latin American countries. It is estimated that 100 million persons are at risk of infection, of whom 16 million to 18 million are actually infected, and there are approximately 50,000 deaths per year due to Chagas' disease. In certain areas of endemicity, approximately 10% of all adult deaths are due to Chagas' disease. In addition to humans, numerous species of wild and domestic mammals can harbor the infection and serve as an enormous parasite reservoir.

The parasite has two stages in the host: the amastigote, the intracellular replicating form, and the extracellular trypomastigote, the infective form (Fig. 32.6). Infection by *T. cruzi* induces a complex immune response that involves effectors and regulatory mechanisms. Control of the infection requires a strong humoral and cellular immune response; hence, the outcome of host-parasite interaction in the early stages of infection is extremely important. During this period of the infection the innate immune response is very important and the macrophage's role in this response is vital. After being phagocytized, the parasite is able to develop intracellularly; however, during later periods, these cells induce its elimination by means of toxic metabolites. In turn, as the infection progresses, adaptive immune response mechanisms are triggered through the Th1 and Th2 responses.

Figure 32.6 *Trypanosoma cruzi*, American trypanosomiasis. (Upper) *Trypanosoma cruzi* trypomastigotes in thin blood films. Note the large kinetoplast (circle); the nucleus tends to be somewhat linear (arrow). (Lower) *T. cruzi* amastigotes in muscle. doi:10.1128/9781555819002.ch32.f6



Immune responses to *T. cruzi* are complex and involve many components, both effectors and regulators. Nonspecific immunosuppression occurs during the first stage of the infection, and the ability to adapt and evade this response allows the organism to invade cells and spread. Thus, the parasite may remain indefinitely in the host's tissues since it is not completely eliminated.

Shortly after infection, both humans and animals develop an immune response to the presence of the parasites; this is clearly demonstrated by the development of parasite-specific antibodies and hypersensitivity reactions. As with many other parasite infections, these immune responses do affect the outcome of the infection; however, host protection is limited. Both antibody-mediated and cell-mediated mechanisms are involved. It has also been shown that autoantibodies directed against laminin, tubulin, and nervous tissue are present; however, whether these autoantibodies contribute to disease is somewhat unclear.

Humoral Immunity

Early in the infection, *T. cruzi* induces an intense inflammatory response, which plays a crucial role in pathogenesis. During the initial phase of the infection, IgM is predominant, whereas later, IgG and IgA become the major antibody classes represented. Antigenic variation, which is characteristic of infections with African trypanosomes, is less common in *T. cruzi* infection. In spite of humoral and cellular immunity to *T. cruzi*, the infection is able to persist in the host.

Reviews of serologic test results show that patients can be divided into four categories: (i) uninfected, (ii) indeterminate (seropositive but asymptomatic), (iii) cardiac (symptomatic with palpitations, dyspnea, syncope, and pulmonary embolus, with findings consistent with chagasic cardiomyopathy), and (iv) mega-gastrointestinal (symptomatic with dysphagia, constipation, and abdominal pain, with documented visceromegaly of the esophagus or colon determined by barium or surgical exploration). Individuals with both cardiac and megagastrointestinal disease were categorized as both (41). There were no significant differences in the levels of antitrypanosomal antibodies among the various clinical groups of chronically infected individuals. Titers obtained by hemagglutination, indirect fluorescent-antibody assay, and enzyme-linked immunosorbent assay allowed a clear distinction between seropositive and seronegative individuals.

The use of recombinant *T. cruzi* antigens greatly improves the early diagnosis of congenital Chagas' disease, especially through the detection of IgM. Although detection of IgM has proven to be very helpful, additional studies related to IgA will be justified, particularly since some congenitally infected infants, who do not produce IgM, may develop specific IgA (42).

Glycoinositolphospholipids are some of the major glycolipids of the *T. cruzi* surface that activate B cells. Apparently, *T. cruzi* glycoinositolphospholipids can directly stimulate NK cells and can induce immunoglobulin production in the absence of T cells and NK cells. These studies indicate that this *T. cruzi*-derived molecule may be one of the stimulators that lead to NK cell activation during infection (43).

An adequate production of proinflammatory cytokines such as IFN-γ, TNF-α, IL-1, IL-12, IL-6, and IL-18 is essential for controlling infection by intracellular parasites. Also, IL-17 may regulate the recruitment of inflammatory cells and the differentiation of Th1 in heart tissue. Therefore, in order to resolve the *T. cruzi* infection, a balance is required between the immune response mediated by Th1 and by Th2. Th1 cells are responsible for the production of inflammatory cytokines, while Th2 cells have an anti-inflammatory function and are involved in the antibody-mediated response. IL-12 and IL-18 produced by dendritic cells and macrophages promote the development of Th1 cells that produce IFN-γ, while IL-4 induces the expansion of Th2 cells and of high amounts of IL-10.

Cellular Immunity

Cell-mediated immunity has also been implicated as a cause of the tissue destruction, including cardiomyopathy and megacolon, seen in Chagas' disease. In animal studies, the transfer of lymphocytes from chronically infected mice to uninfected mice can destroy cardiac cells and the T-cell lines can recreate some of the neuropathologic changes seen in chronic disease (41).

Decreased cell-mediated immune responses have been found in symptomatic patients compared with asymptomatic patients. Future work will probably be focused on T-cell suppression factors or selective cell depletion as possible mechanisms for cell-mediated immunity alterations.

$CD8^+$ T cells are critical to the control of *T. cruzi* infection and function in multiple ways, the most important being the production of IFN-γ; IL-12 is also involved in protection against *T. cruzi* (44–46). This cytokine is produced early by NK cells and later by T cells. Cytokine responses in *T. cruzi*-infected children have been partially clarified; the children were 5 to 14 years of age and were from an area of endemic infection in Paraguay. This age group tends to be the most severely affected by the early phases of infection. Acutely infected children showed a distinct Th1-type IFN-γ cytokine response to infection. The cytokine pattern seen in the seropositive, asymptomatic group was the Th0 type (expression of both IFN-γ and IL-4). It was suggested that selective induction of a Th0-type cytokine pattern is important for the development of cell-mediated and humoral responses that suppress the parasite burden, thus prolonging the onset or limiting the disease severity of chronic Chagas' disease years later (47). Also, the frequency of IFN-γ-producing T cells in patients with chronic Chagas' disease seems to be associated with a history of recent exposure and with the clinical status of the patient (48).

Reduced production of IFN-γ by $CD8^+$ T cells is associated with increased severity of Chagas' disease in humans. In chronic infection, $CD8^+$ T cells serve as effector memory cells, undergo rapid expansion, and demonstrate effector functions following reexposure to antigen. However, these responses appear to develop relatively slowly, and the effector function of the $CD8^+$ cells is compromised in some tissues, including muscle (45).

In several patients coinfected with *T. cruzi* and HIV with high parasitemia, no clinical or parasitological evidence of *T. cruzi* reactivation was seen. $CD4^+$ T-cell counts decreased over time in two patients, and the lymphocyte proliferative response to the parasite was very low in all patients. Thus, *T. cruzi* infection appears to have a long silent course in immunocompromised HIV-infected patients (49).

In a group of 23 patients with AIDS and *T. cruzi* infection, 20 patients developed severe multifocal or diffuse meningoencephalitis with necrosis and hemorrhage associated with numerous tissue parasites (50). The second most severely affected site was the heart, with myocarditis in 7 of the 23 patients. Both acute myocarditis and meningoencephalitis are thought to be caused by relapses of chronic *T. cruzi* infection, but this hypothesis is not universally accepted. Since immunologic defense against *T. cruzi* is mediated mainly by T lymphocytes and since the $CD4^+$ population of T cells is depleted in AIDS patients, it appears in certain circumstances that AIDS may be responsible for reactivation of *T. cruzi* infections. In patients who do not have AIDS but have fatal, acute infections with *T. cruzi*, meningoencephalitis is uncommon and is seen most often in children younger than 4 years. Cases of Chagas' disease in immunosuppressed individuals have been described as having pseudotumoral presentations. There are expanding mass lesions in the cranial cavity, causing intracranial hypertension, and they mimic various neoplasms such as gliomas, lymphomas, or metastases (50). The United States, with the largest HIV-infected population reported by the

World Health Organization (WHO) up to 1987, is also known to have a significant number of individuals with Chagas' disease (51).

Studies of IL-12 indicate that this cytokine can act as a more selective amplifier of *T. cruzi* reactive cells than IL-2 does. By enhancing parasite antigen-specific immunity, IL-12 could be potentially therapeutic, with the ability to control reactivated *T. cruzi* infections associated with AIDS or other immunosuppressive conditions (52).

Macrophages play an indispensable role in the primary response to pathogens but also take part in the resolution process of the inflammation and homeostasis of tissues. These cells are activated by IFN-γ and TNF-α (type 1), or by an alternative way stimulated by IL-4 and IL-13 (type 2) (53). Classically activated macrophages are currently grouped in group M1, alternatively activated ones are in M2a, those that polarize towards a Th2 response are designated M2b, and those whose stimulation is mediated by glucocorticoids and transforming growth factor β (TGF-β) are placed in group M2c.

Immunosuppression

Suppressed delayed-type hypersensitivity has been reported to occur during acute Chagas' disease. *T. cruzi* also exerts a direct suppressive effect on human lymphocytes. Abnormalities in activated human lymphocytes may be involved in immunosuppression. These changes occur regardless of whether the parasites and cells are in physical contact. There is also evidence that a soluble *T. cruzi* product mediates the suppressive effects. This trypanosomal immunosuppressive factor is a protein and can be purified from sera from chronic chagasic patients but not healthy individuals (48).

B cells isolated at the acute stage of infection have shown marked impairment in their response to polyclonal activators *in vitro*. Studies suggest that B-cell apoptosis and cell cycle arrest could be the mechanisms that control intense B-cell expansion but at the same time could be delaying the emergence of a specific immune response to *T. cruzi* (54).

Splenomegaly, lymphadenopathy, depression of immune system responses to unrelated antigens, hypergammaglobulinemia, and autoimmunity are all indications of immune system physiopathologic changes in Chagas' disease. Minoprio et al. (55) also think that specific antigenic challenges could elicit "nonspecific" immune responses greater than the antigen-specific components of the response. Underlying mechanisms might include, for example, the production of cytokines, the nonspecific "stickiness" of antigens to lymphocyte surfaces, the expression of major histocompatibility complex and other cell surface components (probably IFN-γ and IL-4), and the nonspecific lymphocyte mitogens released by *T. cruzi* or superantigens which could bind to T-cell receptors and trigger accessory cell-dependent T-cell activation.

Studies have shown that there appears to be no correlation between NK and lymphokine-activated killer (LAK) cell functions in the same individual. Neither NK nor LAK cell functions seem to be involved in the immunosuppression associated with chronic Chagas' disease (56).

Autoimmunity

Chagas' disease cardiomyopathy (CCC) is one of the few examples of postinfectious autoimmunity, where *T. cruzi* triggers molecular mimicry-related target organ immune damage. Heart tissue destruction in CCC may be caused by autoimmune recognition of heart tissue by a mononuclear cell infiltrate, often many years after the initial infection. Indirect evidence indicates that there may be cross-reactivity between *T. cruzi* and cardiac tissue. There is also evidence for immune system recognition of cardiac myosin in CCC; the presence of an anti-cardiac myosin cross-reactive *T. cruzi* antigen is suspected as the cause. Studies demonstrate sequence homology to a recombinant *T. cruzi* protein (B13) in several positions of cardiac myosin heavy chain. This recognition of a heart-specific *T. cruzi* cross-reactive epitope in close association with the presence of chronic heart lesions suggests the involvement of cross-reactivity between cardiac myosin and B13 in the pathogenesis of CCC (57, 58). It has been proposed that phagocytic removal of infected apoptotic cardiomyocytes, combined with signaling through innate immune receptors, may be required to initiate immune responses that damage the heart (59).

Studies demonstrate the presence of antibody to myelin basic protein in the serum of mice chronically infected with *T. cruzi.* Studies of the overlapping peptides from the myelin basic protein identified two regions responsible for the cross-reactivity with *T. cruzi* (60). This may provide further evidence to help explain why chronic Chagas' disease is characterized by inflammatory infiltrates in myocardial and nervous tissues, with virtually no demonstrable parasites.

Another study suggests that fraction IV *T. cruzi* (parasite antigen acidic fraction) and sciatic nerve components have some epitopes, possibly carbohydrate, in common. Thus, infection in patients with Chagas' disease could override tolerance to self components and lead to autoimmunity (61). The observation of potentially shared epitopes between some of the parasite and cardiac proteins has led to the popular idea of cross-reactive proteins to explain autoimmunity. Notably, the B13 epitope of *T. cruzi* has been reported to share peptide sequence with cardiac myosin (62).

The scarcity of parasites in the chronic phase of the disease contrasts with the severe cardiac pathology seen in approximately 30% of chronically infected patients. Antigen-specific and non-antigen-specific mechanisms have been suggested to explain T- and B-cell activation leading to autoimmunity. However, the issue of autoimmunity versus parasite persistence as the cause of Chagas' disease pathology continues to be hotly debated. Autoimmune and *T. cruzi*-specific innate or adaptive responses are not incompatible or mutually exclusive, and it is likely that a combination of both is involved in the pathogenesis of chronic Chagas' disease cardiomyopathy.

Malaria

Human malaria is caused by infection with any of five species of *Plasmodium*, with *Plasmodium falciparum* and *Plasmodium knowlesi* being the most pathogenic. The other three species are *P. vivax*, *P. ovale*, and *P. malariae* (Fig. 32.7). Symptoms of malaria infection include fever and malaise; infections with *P. falciparum* or *P. knowlesi* can be fatal. This is particularly true in untreated, nonimmune individuals. Despite the high fatality rate in nonimmune or partially immune individuals, people who live in areas of endemic infection for long periods generally acquire immunity and may be asymptomatic or have very mild symptoms. Why and how this slow development of immunity occurs is not completely understood, particularly when reviewing the very complex life cycle of these organisms.

The vector for malaria is the female anopheline mosquito. When the vector takes a blood meal, sporozoites contained in its salivary glands are discharged into the puncture wound. A surface protein, the circumsporozoite protein, in combination with thrombospondin-related adhesive protein allows recognition of glycosoaminoglycans in the liver, thus providing the targeting mechanism that allows the *Plasmodium* sporozoite to reach the correct liver cells. Within an hour, these infective stages are then carried via the blood to the liver, where they penetrate parenchymal cells and begin to grow, thus initiating the pre-erythrocytic or primary exoerythrocytic cycle. The sporozoites become round or oval and begin dividing repeatedly, resulting in large numbers of exoerythrocytic merozoites that leave the liver and invade the red blood cells (RBCs) (erythrocytic cycle). In *P. vivax* and *P. ovale*, a secondary or dormant schizogony may occur from organisms that remain quiescent in the liver until a later time (hypnozoites). Merozoite surface proteins facilitate recognition of and adhesion to the RBC.

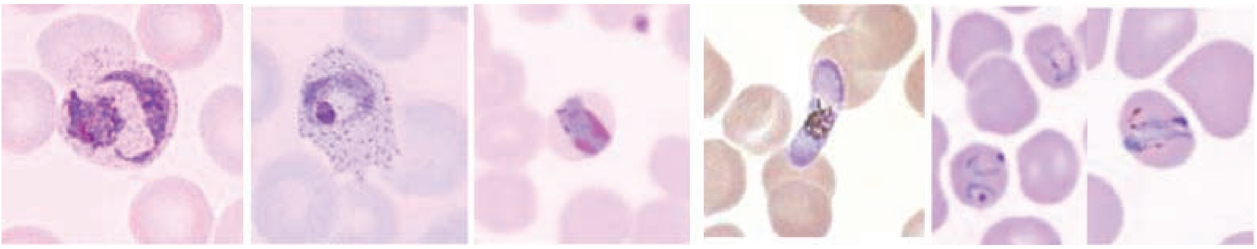
The situation in which the RBC infection is not eliminated by the immune system or by therapy and the number of RBCs begins to increase again with subsequent clinical symptoms is called a recrudescence. All species may have a recrudescence. The situation in which the erythrocytic infection is eliminated and a relapse occurs because of a new invasion of the RBCs from liver merozoites is called a recurrence or true relapse and theoretically occurs only in *P. vivax* and *P. ovale* infections.

Once the erythrocytes and reticulocytes have been invaded, the parasites grow and feed on hemoglobin. As the nucleus begins to divide, the trophozoite is called a developing schizont. The mature schizont contains merozoites, whose number depends on the species, which are released into the bloodstream and invade RBCs, in which a new cycle of erythrocytic schizogony begins. After several erythrocytic generations, some of the merozoites begin to develop into the male and female gametocytes.

The asexual and sexual forms just described circulate in the bloodstream in infections with four species of *Plasmodium*. However, in *P. falciparum* infections, as the parasite continues to grow, the RBC membrane becomes sticky and the cells tend to adhere to the endothelial lining of the capillaries of the internal organs. Thus, only the ring forms and the gametocytes (occasionally mature schizonts) normally appear in the peripheral blood.

If gametocytes are ingested when the mosquito takes a blood meal, they mature into gametes while in the mosquito gut. The male microgametes undergo nuclear division (exflagellation), in which they break out of the RBC, become motile, and penetrate the female macrogamete; the fertilized stage is then called the zygote. The zygote becomes elongate and motile and is called the ookinete; it

Figure 32.7 *Plasmodium* spp., malaria, representative typical stages for each of the five human species. (Left to right) *P. vivax* developing trophozoite in enlarged RBC; *P. ovale* developing trophozoite with RBC fimbriated edges; *P. malariae* band form in normal/small RBC; *P. falciparum* crescent-shaped gametocyte; *P. knowlesi* double rings/band form, resembles mix of *P. falciparum* and *P. malariae*. doi:10.1128/9781555819002.ch32.f7



migrates to the mosquito midgut, secretes a thin wall, and grows into the oocyst, which extends into the insect's hemocele. Within a few days to 2 weeks, the oocyst matures and hundreds of sporozoites are formed. When the oocyst ruptures, the sporozoites are released into the hemocele and dispersed throughout the body, and some make their way into the salivary glands. When the mosquito next takes a blood meal, the sporozoites are injected with saliva into the host.

Each phase of the life cycle is associated with the expression of many stage- and species-specific proteins, many of which are found within the surface membrane of the parasite. These surface proteins are highly polymorphic and antigenically variable. Two hypotheses have been developed to explain the slow development of immunity: (i) immunity is strain specific, and so the individual would not become immune until exposed to all the major antigenic variants circulating within the area of endemicity, and (ii) acute malaria causes extreme immunosuppression, which delays the development of protective immunity. Also, antidisease immunity may be very different from antiparasite immunity. Even continuous exposure to malaria parasites rarely produces sterile immunity, and blood-stage parasites can be detected by PCR in all age groups (63). The wide range of malarial surface antigens helps explain why the development of clinical immunity requires years of repeated infections. Two mechanisms contribute to antigenic diversity, one being the genetic nucleotide replacement and recombination and the second being antigenic variation. Since different antigens are expressed during various parts of the life cycle, naturally acquired immunity tends to be stage specific. Both humoral and cellular components are required for naturally acquired immunity. CD4$^+$ and CD8$^+$ T cells are effective against intracellular liver-stage organisms, while antibodies may interfere with host cell invasion by sporozoites and merozoites. Also, antibodies to variant surface antigens expressed on the RBC surface may facilitate phagocytosis of the infected cells and prevent their adhesion to endothelial receptors.

Apparently, internal antigens are less variable and are partially conserved among different malarial species. When released during the rupture of mature schizonts, these internal antigens appear to stimulate the cytokine cascade, which leads to various symptoms and pathologic changes (64–70) (Table 32.13).

Humoral Immunity

Malarial infection leads to production of high levels of TNF and other proinflammatory cytokines; other findings include elevated immunoglobulin production, activation of complement, and redistribution of lymphocytes from the peripheral circulation to the spleen and other organs. Antibodies can confer protection against *P. falciparum* and *P. vivax*; neonates and infants are protected by maternal antibodies and, in clinical treatment trials, by immune serum or purified immunoglobulins. Animal studies involving passive transfer of whole serum or purified IgG can have a dramatic impact on the erythrocytic cycle by extending the prepatent period, reducing the parasitemia, and leading to spontaneous cure.

Levels of antimalarial antibodies in humans increase during childhood, become maximal during early adulthood, and subsequently remain fairly constant. However, there appears to be little correlation between antibody levels and protection against infection or clinical disease. Also, some of the antibodies are not directed against *Plasmodium* organisms. These antibodies may represent the degree of past exposure rather than functional immunity.

CD4$^+$ T cells are the major source of both IFN-γ and TNF-α, which are implicated in both protection and pathology of this disease. It may be that antibodies and CD4$^+$ T cells provide protection against symptomatic malaria but CD8$^+$ T cells are required for sterile immunity (71).

Cellular Immunity

Animal studies indicate that T-cell-dependent immune mechanisms are required for the development of effective immunity. Although animal model results vary and may not always mimic human immune responses, it does appear that the human antimalarial response is both T-cell dependent and independent. It appears that intrahepatic CD8 T central memory cells, which are maintained in part by liver stage Ag depot and by IL-15-mediated survival and homeostatic proliferation, form a reservoir of cells ready for conscription to CD8 T effector/effector memory cells needed to prevent reinfections (72).

In the erythrocytic portion of the parasite life cycle, T cells are thought to serve at least two functions in immunity: (i) assisting in antibody production and (ii) producing cytokines. The role of cytokines in malaria is very complex; they affect parasite growth and clearance, and they activate macrophages to engulf infected RBCs and to kill the malarial parasites. Elevated concentrations of TNF, IL-6, and soluble TNF receptors have been found in serum in patients with severe *P. falciparum* infections.

TNF induces nitric oxide (NO) production. NO definitely plays protective roles in malaria: (i) it acts as a relaxing factor; (ii) it can downregulate cell adhesion molecules; (iii) it can reduce leukocyte-endothelial cell adherence; (iv) it can scavenge free oxygen radicals; (v) its inhibition can cause endothelial cell alterations, such as albumin leakage and platelet deposition; and (vi) it can reduce platelet aggregation and adhesion to endothelium (73). The role of NO in pathogenesis is somewhat less clear. It is possible

TABLE 32.13 Updated information on immune responses seen in malaria infections[a]

Immune response	Comments	Reference
Parasite antigen-specific IL-10 and antibody responses predict accelerated parasite clearance in *P. falciparum* malaria	Parasite antigen-specific IL-10-mediated antibody responses may play a role in the control of asexual-stage parasite multiplication	64
P. falciparum merozoite surface protein 1 antigen (MSP119): induction of small T-cell response (IL-4), significantly increased by addition of IL-2; if IFN-γ, IL-12, or neutralizing anti-IL-4 antibody is present, T-cell responses are abolished	Recombinant MSP119 is a leading malaria vaccine candidate, can prime nonimmune human lymphocytes	65
Correlation of T-cell response and lymphokine profile with RESA peptides of *P. falciparum* containing a universal T-cell epitope and an immunopotentiator, polytuftsin	Cytokine profile is suggestive of a $CD4^+$ Th1 type of immune response; ideal for killing intracellular pathogens like plasmodia	66
TNF-α, IL-1β, IL-6 implicated in pathogenesis of severe *P. falciparum* malaria; exogenous IL-10 inhibited malarial antigen-induced cytokine production; maximal inhibition when IL-10 used in first 2 h of stimulation of PBMC	TNF-α, IL-1β, IL-6 produced within 2–4 h of stimulation; IL-10 produced after 8 h; findings suggest that IL-10 counterregulates proinflammatory response to *P. falciparum;* severe falciparum malaria may be associated with inadequate negative feedback response by IL-10	67
IFN-γ responses associated with resistance to reinfection with *P. falciparum* in young African children	Differences in Th1/Th2 cytokine balance may be linked to ability to control parasite multiplication, helping explain marked differences in both susceptibility to infection and clinical presentation	68
PfEMP-1 molecule expressed on surface of RBC infected with trophozoite or early gametocyte parasites; IgG antibodies to PfEMP-1 increased with age, mirroring decline in both prevalence and density of asexual stages and gametocytes in RBCs	Immunity to PfEMP-1 may influence malaria transmission by regulation of gametocyte production; mechanisms may be (i) controlling asexual proliferation and density and (ii) affecting gametocyte maturation	69
Cell-mediated immunity and cytokines involved in pathogenesis of malaria; MM and CM cases in children monitored	Both groups (MM, CM) had significantly higher levels of IL-6, IL-10, and TNF-α than did controls; 24 h after admission, IL-10 and IL-6 levels much higher in CM patients; findings suggest activation of monocyte-macrophage system during early stage of clinical malaria	70

[a] CM, cerebral *P. falciparum* malaria; MM, mild *P. falciparum* malaria; PBMC, peripheral blood mononuclear cells; PfEMP-1, *P. falciparum* erythrocyte membrane protein 1; RESA, ring-infected erythrocyte surface antigen.

that NO can mediate early changes in cerebral malaria, such as neurotransmission disturbances, but NO is probably not involved in the actual process of neurovascular damage.

Studies suggest that a systemic, excessive production of TNF, not compensated for by TNF receptors or a hyperproduction affecting vulnerable sites such as the brain, may exacerbate the disease and lead to vascular abnormalities and life-threatening malaria. On the other hand, TNF produced within the context of a specific immune response may be the major protective cytokine in malaria infections. This demonstrates the dual role of this particular cytokine (74).

Although many of the published research reports indicate that a number of monokines and lymphokines are released during acute malaria infections, interpretation of the relevance of cytokine levels may be somewhat misleading (75). Summary information related to the elimination of infected RBCs and mechanisms by which the parasite evades the host immune system can be seen in Table 32.14 (76, 77).

Immunosuppression

It has been well documented that acute malaria infections lead to a decrease in host immune system functionality. These patients have been described as being temporarily immunosuppressed. Direct evidence for this decreased immune system capability has been summarized by Riley et al. (78) and includes increased susceptibility to bacterial infections, reactivation of latent viral infections, and diminished response to some vaccines in children. Indirect evidence is that children protected by chemoprophylaxis for malaria are less susceptible to other infections and tend to respond better to routine vaccinations (78). Although this evidence indicates a lowered immunocompetency level in patients with malaria, it is not known exactly which factors influence this change. It may result directly from the level of parasitemia or from the overall impact of a febrile illness, such as malaria.

During acute malaria infections, there is a decrease in the response to parasite antigens. This may be due to immunosuppression, discussed above, or may just indicate a lack of "priming of the system" in patients with no prior exposure

TABLE 32.14 Mechanisms of infected RBC elimination and evasion of the host immune system in *P. falciparum* infections[a]

Mechanism	Occurrence in *P. falciparum* infection
Infected RBC elimination	
Opsonization	Yes
Antibody-dependent cell-mediated toxicity	Yes
NK cell activity	Yes
Cytotoxic T-cell activity	No
Evasion of host immune system	
Infected RBC knob-like membrane changes	Yes
Infected RBC surface antigenic changes	
Modified host antigens	Yes
Parasite-derived antigens	Yes
Clonal antigenic variation	Yes
Cytoadherence	
Endothelial	Yes
Autoagglutination	Yes
Rosetting of both infected and normal RBCs	Yes
Upregulation of host ligands	Yes

[a] Data from references 76 and 77.

to the organism. Therefore, increased immune responses after cure may be due to decreased immunosuppression or increased levels of preexisting immune responses. Overall, the literature suggests that in some settings T regulatory cells might modulate detrimental inflammation involved in disease induction, while in other cases T regulatory cell-mediated inhibition of immune responses results in increased parasite density, which is also known to be a key determinant of disease severity (79).

Several factors have been described as virulence factors with *P. falciparum*; however, formal documentation that these characteristics are always associated with virulence and significantly more likely to cause severe disease is not abundant (77). These factors include the following: (i) the multiplication capacity in which the biomass of parasites may outstrip host defense mechanisms; (ii) the RBC selectivity limiting the overall number of infected RBCs (however, this is not a big factor for *P. falciparum*); (iii) the cytoadherence and rosetting ability where all five species can cause rosetting but only *P. falciparum* can cause cytoadherence, which is thought to be linked to cerebral malaria; (iv) the potential to release cytokines; (v) antigenicity (those not recognized by the host will have an advantage); and (vi) antimalarial drug resistance, with a great deal of selective pressure against drug-sensitive parasites (77).

Numerous studies seem to emphasize that the host immune system has difficulty in controlling the infection; many unusual host responses have been described. These unusual responses may be due to host immune system overreaction, leading to inappropriate responses. The difference between protective immunity and pathology is a very delicate balance and one in which specific mechanisms are difficult to clarify (80). Specific immunopathologic complications of malaria infection are listed in Table 32.15 (81–86).

Malaria Vaccines

Pre-Erythrocytic and Erythrocytic Vaccines. With a continuing lack of a highly effective vaccine candidate against *P. falciparum* malaria, it remains imperative that all options be explored that could lead to the successful development of such candidates. The development of a subunit vaccine targeting the asexual blood stage of *P. falciparum* malaria infection has been problematic, with only limited success in clinical trials. However, only a fraction of potential blood-stage vaccine antigens have been evaluated as targets, and a number of new promising candidate antigen formulations and delivery platforms are approaching clinical development. There are two main targets of these studies: (i) antigens that are associated with the merozoite's surface or its organelles (pre-erythrocytic antigens), and (ii) proteins that the intracellular parasite exports to the surface of the infected RBC. However, because merozoites are free in the circulation for minutes only, this defines important requirements for the functional quantity and quality of antibodies required for parasite elimination. On the other hand, *P. falciparum* exports between 200 and 400 proteins through the parasitophorous vacuole membrane into the RBC, and some are localized on the RBC plasma membrane. There is also evidence that other gene products such as rifins, stevors, and surfins are also present on the RBC membrane; however, their functional roles are not clearly defined. Major limitations on the development of a merozoite vaccine include the antigenic polymorphism of important targets and problems in producing these molecules in a native configuration. In addition, only a few potential vaccine targets have been investigated, and there is a lack of *in vitro* assays that correlate with immunity *in vivo* and a limited understanding of the types of immunity required. There remain very good reasons to pursue development of vaccines targeting the blood stages, including (i) naturally acquired immunity arises against the blood stages, (ii) an effective blood-stage vaccine could reduce disease severity and mortality, and (iii) this type of vaccine could potentially reduce malaria transmission by reducing gametocyte densities (Table 32.16) (87, 88).

Sexual Stage/Transmission-Blocking Vaccines. Transmission-blocking vaccines, which target the sexual stages of malaria parasites to interfere with and/or block the parasite's development within mosquitoes, have been regarded

TABLE 32.15 Immunopathological complications of malaria infection[a]

Pathology	Suspected mechanism	Comments
Hypergammaglobulinemia	Antigen-induced cytokine production (IL-6); antigenic variation; polyclonal activation	IL-6 from fibroblasts, macrophages, and tumor cells; increases synthesis and secretions of immunoglobulins by B cells; most antibodies not directed against malaria antigens; common feature of antigenic variation is presence of successive peaks of infection, each new peak being antigenically different from the previous one; partial loss of suppressor mechanisms coupled with normal CD4 helper/inducer activity may result in high levels of immunoglobulin in serum (characteristic of people living in areas of endemic malarial infection)
Immunosuppression	Macrophage dysfunction; antigenic competition; structural disruption of germinal centers; disruption of spleen function; polyclonal activation and immune system "exhaustion"	May be due to suppressive effect of malarial pigment ingested; defects in monocyte and neutrophil chemotaxis, reduced monocyte phagocytic function
Nephrotic syndrome	Immune complex deposition; autoimmunity	In nephrotic syndrome associated with *P. malariae,* malaria antigen and immune complexes can be eluted from the kidneys
Autoimmunity	Autoantibodies; antierythrocyte antibodies; dyserythropoiesis; excessive erythrophagocytosis	Defective IL-12 production in early course of infection may result in fatal anemia; destruction of uninfected RBCs occurs through phagocytosis of RBCs bound to merozoites killed as a result of malaria paroxysms (thus, dyserythropoiesis may play a minimal role in anemia); hemolysis is prime cause of anemia, although destruction of parasitized RBCs is not the sole cause of the hemolytic process; bone marrow suppression may be insignificant, but preexisting iron deficiency aggravates the severity of anemia; immune recognition of "self" or "modified self" RBC antigens by B or T lymphocytes may occur
Thrombocytopenia	Excessive removal of platelets; coating of platelets with malaria antigen	May also be due to platelet inactivation; increased platelet turnover; platelet-bound antibody controversial
Hyperactive malarial splenomegaly	Genetic predisposition; hypergammaglobulinemia; chronic increase of lymphocyte proliferation	Associated with increased capacity to clear RBCs by Fc receptor-mediated mechanisms and recognition of reduced deformability; spleen may also modulate cytoadherence
Burkitt's lymphoma	Coendemicity with EBV; polyclonal activation; antigen-induced cytokine production	In patients with *P. falciparum* malaria, loss of cytotoxic T-cell control of EBV in B cells, possibly due to destruction or dysfunction of a subset of CD4 cells responsible for induction of suppressor/cytotoxic CD8 cells, leads to activation and proliferation of foci of B cells containing EBV; the expanded pool and rapid turnover of these cells may increase the chances of malignant transformation, leading to the genesis of Burkitt's tumor

[a]Adapted from references 81–86. EBV, Epstein-Barr virus.

as important targets for disrupting the malaria transmission cycle. Transmission-blocking vaccines provide a promising strategy for controlling *P. vivax*, since this parasite has a unique biological characteristic in which it undergoes early gametocytogenesis prior to the manifestation of clinical symptoms, which enables parasite transmission prior to treatment (89). A proposed target profile for a malaria sexual stage and mosquito transmission-blocking vaccine can be seen in Table 32.17 (90).

A set of conserved "invasion receptors" in the midgut of diverse anopheline species are used by *Plasmodium* ookinetes to attach to the midgut epithelium. Antibodies directed against such receptors have been shown to block development of oocysts in membrane-feeding transmission-blocking assays. A vaccine based on such conserved vector antigens should be effective against all species of *Plasmodium* and would eliminate the need to develop separate vaccines for different *Plasmodium* species. Moreover, since such vaccines target vector antigens, parasite strain diversity, which has been a major problem for malaria vaccine development, will be overcome.

Other factors related to gametocytes will need to be considered. First, the gametocyte reservoir is much larger than suggested by blood film examination, and transmission can occur from individuals with very low numbers of gametocytes. Gametocyte infectivity is related to gametocyte density; however, small numbers of infectious gametocytes in adults are as important in maintaining transmission as larger numbers of gametocytes in children susceptible to clinical attacks of malaria.

TABLE 32.16 *Plasmodium* vaccine development: continuing problems[a]

Problem	Comments
Baseline studies	Detailed studies required: burden of disease, clinical spectrum, incidence of severe malaria and mortality; relapse patterns, strain heterogeneity, interactions with other species; mixed infections; economic analyses
Polymorphisms	The existence of many polymorphisms in antigens selected for vaccine development requires new immunologic strategies for eliciting cross-reactive immune responses. Another option would be the design of large constructs consisting of multiple alleles of variant antigens.
Antibody titers	Need for extremely high antibody titers for anti-merozoite antigen vaccines to achieve protection
Protective immune responses	Knowledge is limited regarding the full range of protective immune responses to the parasite developmental stages. Need to clarify possible effector roles for $CD4^+$ T cells against pre-erythrocytic and erythrocytic antigens
Consensus	Lack of consensus regarding mechanisms of blood-stage immunity and vaccine design (e.g., antibody versus T cell-inducing)
Adjuvants	Lack of safe, well-defined adjuvants
Potential vaccine candidates	Less than 1% of parasite genes have been explored in the context of vaccine candidates from all parasite developmental stages.
Screening methods	Lack of reliable screening methods for selection of promising vaccine candidates
Recombinant expression systems	Need for recombinant expression systems capable of producing plasmodial proteins like that found in the native parasite molecule. Need comprehensive structural studies to define native antigen structures.
P. vivax vaccine	Very limited attention to vaccine development for *P. vivax*, the cause of significant worldwide morbidity. Potential problems include: • Gametocytes appear early in the infection • Persisting hypnozoites in the liver • Extrinsic cycle is shorter, completed at lower temperature • Efficient transmission even when mosquitoes are highly seasonal • *P. vivax* and *P. falciparum* exist in same geographic area • Data on sensitivity to drugs, especially primaquine (issues of primaquine tolerance and resistance), rate of G6PD deficiency.

[a] Adapted from references 3, 87, and 88.

Helminth Infections

Helminths are multicellular parasitic worms belonging to the nematodes (round worms), trematodes (flukes), and cestodes (tapeworms), which account for half of the WHO-designated “Neglected Tropical Diseases” and infect 1 billion to 2 billion of the world’s poorest people, with soil-transmitted helminths (gastrointestinal nematodes including *Ascaris*, *Trichuris*, and *Necator* sp.) and schistosomes (blood-dwelling trematodes) being the most common. Although great strides have been made through implementation of chemotherapy and improved sanitation, massive amounts of suffering due to helminth infections persist, and to date, no vaccines for helminths exist. Although this is probably a gross underestimation of the total, the estimated disease burden of helminth infection is at least 13,000,000 DALYs (years of life and productive life lost due to disability and/or death).

Most species do not multiply in the human host, and helminth infections increase through repeated transmission, confirming chronic susceptibility and parasite longevity. In general, the Th2 arm of the cellular immune system tends to guide the antihelminth immune response, acting through inducer and activating cytokines including Il-4, IL-5, IL-9, and IL-13. These mediators promote effector mechanisms ranging from antibodies, macrophages, and granulocytes to epithelial cell responses that tend to be less prominent in protozoal infections.

Nematodes are protected by a collagen layer that comprises the cuticle, which is overlain by a lipid-rich epicuticle and a glycoprotein surface coat. The cuticle is resynthesized and shed every time the worm enters a new developmental stage. The surface of the schistosome (trematode) is complex and varies throughout its life cycle. The outer layer consists of a tegument, a syncytial layer of cells that is bounded apically by a complex invaginated membrane. The tegument is composed of secreted lipid-rich “membranocalyx,” as well as “glycocalyx,” the latter of which is partially discarded upon transformation of cercariae to schistosomules, but also appears to be prominent on the surface of adult worms. The expression of both proteins and glycans is regulated from one life stage to the next and is highly variable.

TABLE 32.17 Proposed target profile for a malaria sexual stage and mosquito transmission-blocking vaccine[a]

Target	Comments	Issues
Indication	Prevention of transmission of *P. falciparum* or *P. vivax* (including hypnozoites)	*P. falciparum* candidates: • Gametocytes • Gametocytes and gametes • Zygotes and ookinetes *P. vivax* candidates: • Gametocytes • Gametocytes and gametes • Zygotes and ookinetes
Efficacy	>85% efficacy and reduction of the CRR below 1	Possibilities based on: (i) transmission blockage can be achieved by human immune mediators, such as immunoglobulins or cytokines, which are induced by parasite components expressed on the sexual parasite forms exposed to the human immune system during their circulation in the human body; and (ii) as observed in nature, malaria infections are able to boost primary antibody responses against gametocytes and increase the TB effects of the corresponding sera. Immunogenic proteins that are important for parasite fertilization or other vital functions of the parasite sexual forms are considered targets for TB vaccine development. Using other control tools, reduces CRR to below 1.0 for at least a year.
Duration	Effective for at least 2 yr	Need to focus on transmission impact rather than morbidity/mortality alone; may actually contain many life cycle targets (pre-erythrocytic, erythrocytic, sexual stages)
Safety	Vaccine has a safety and efficacy profile comparable to hepatitis B vaccine	Can be safely administered to pregnant women; should be no increased risk of autoimmune or other chronic diseases related to vaccination
Target population	All age groups, including pregnant women, people with immunodeficiencies, malnourished individuals, high-risk populations	May be limited to otherwise healthy people (infants, children adolescents, adults in areas of malaria endemicity)
Administration	Orally or by intramuscular of subcutaneous injection or other innovative device	Oral provides needle-free vaccination. BMP elicits type 2 response; BNP elicits type 1 response; BNP or BMP bypasses issue of tissue barriers and proteolytic environments; some studies in mice indicate blocking antibody activity of 99% with no significant differences in routes of immunization
Schedule	A single-dose schedule that can be administered by mass administration or clinic-based programs. May require booster dose after 2 yr. Minimal schedule is 3 doses, administered over 6 mo	Booster may actually be necessary 4–6 mo after the second dose and after 2 yr.
Stability, storage	Minimum shelf life 36 mo, stored at ambient temp; withstand freeze/thawing; minimum stability 30 min at 37°C, 2 yr at 2–8°C	Shelf life of at least 24 mo; vaccine vial monitor should be attached
Coadministration	No interference or interactions with other vaccines expected to be concurrently administered	Vaccine may need to be given as stand-alone product not coadministered with other vaccines.

[a] Adapted from references 89, 90, 100, and 101. BMP, biodegradable microparticle; BNP, biodegradable nanoparticle; CRR, case reproductive rate (number of individuals who can be infected from a single untreated malaria case in an endemic area); TB, transmission blocking.

TABLE 32.18 Immune responses to parasites (protozoa and helminths)[a]

Parasite or infection	Comments
Protozoa	
Intestinal protozoa *E. histolytica*, *G. lamblia*	Most organisms in gastrointestinal tract are nonpathogenic, i.e., do not elicit immune responses. *Overall immune response:* little correlation between antibody levels and protection; repeated invasion of gut wall (amebiasis) relatively common, reinfection rare in hepatic amebiasis
Leishmaniasis Self-healing dermal forms (*L. tropica*, *L. mexicana*) Nonhealing visceral forms (*L. donovani*)	In addition to two general categories, wide spectrum of disease, immune response slow to develop (few months to >1 yr); strong immunity to homologous strain, little antibody produced; visceral infections frequently progress to death; ~25% of patients do recover *Overall immune response:* cell mediated; parasite killing is intracellular and involves production of toxic metabolites by host cell; no apparent role for antibody or complement
African trypanosomiasis	Successive waves of parasitemia; antigens differ in each wave (antigenic variation); immune response rapid, efficient *Overall immune response:* agglutination, complement-mediated lysis; cell-mediated responses involved to a lesser degree
American trypanosomiasis	Incubation, 2–3 wk; acute phase, 4–5 wk; chronic phase, ≥20 yr; latent phase/no symptoms, ≥20 yr; spontaneous recovery rare (some individuals die soon after infection) *Overall immune response:* antibody involved; immunopathology is a very important aspect.
Coccidiosis	Self-limiting, immune response not that important in control but very effective against reinfection with homologous organism *Overall immune response:* antibody-mediated response probably most important; dramatic differences between immunocompetent and immunosuppressed hosts (*Cryptosporidium* spp.)
Toxoplasmosis	Antibody controls infection but will not eliminate residual organisms; no protection against reinfection, but severity reduced *Overall immune response:* probably macrophage activation reinforced by antibody; dramatic differences between immunocompetent and immunosuppressed hosts
Malaria	Time span, 2–4 yr (*P. vivax*, *P. ovale*, *P. falciparum*, *P. knowlesi*) or lifelong (*P. malariae*) *Overall immune response:* no immune response to exoerythrocytic stages; immunity does occur to homologous strains; antibody transfer effective (mother to child); strong correlation between antibody and parasite disappearance; antibodies stage specific; T cells probably serve at least two functions (assisting in antibody production and producing cytokines).
Babesiosis	Pathogenesis not fully understood; lasting immunity after infection, antibody mediated; splenic immune properties unrelated to specific acquired immunity *Overall immune response:* humoral system-mediated immune response present; impact on host cell-mediated response more important; activated macrophages important; also evidence for some immunosuppression of the host but may not be relevant to pathogenesis
Helminths	
Schistosomiasis	No evidence of spontaneous recovery, can resist infection *Overall immune response:* primary response against skin penetration by schistosomula, effective against reinfection; six methods have been identified: (i) IgG + neutrophils, (ii) IgG + eosinophils, (iii) IgG + macrophages, (iv) IgG + eosinophils + mast cells, (v) IgE + macrophages, (vi) IgE + eosinophils + mast cells *In vitro* studies indicate that eosinophils bind to schistosomula, extrude hydrolytic enzymes onto surface of worm, disrupt membrane, allow eosinophils into body, and detach tegument; macrophages become involved after damage by eosinophils and neutrophils; mechanisms probably similar in human disease. *Most important components:* hypersensitivity responses are initiated by eggs trapped in the tissues (granulomas and local tissue damage, particularly in the liver); blockage of blood flow can cause portal hypertension and vascular abnormalities.
Cestode infections	Adult forms show no evidence of acquired immunity; in larval forms, immunity is slow to develop. *Overall immune response:* larval forms evade immune response; animal studies indicate eosinophils and mast cells involved in damage to cyst, with subsequent encapsulation by various cells including macrophages; early part of immune response may be similar to that seen in schistosomiasis; larval forms exert immunosuppressive effects on the host such as larval secretions inhibiting T-cell proliferative responses and IL-2 production, preventing accumulation of inflammatory cells, and interfering with normal macrophage functions

(continued)

TABLE 32.18 *(continued)*

Parasite or infection	Comments
Intestinal nematodes	Strong immune response to all intestinal nematodes (elicited by migrating larvae); self cure (spontaneous elimination of adult worms in gastrointestinal tract); worm infections elicit Th2-subset responses, cytokines generate inflammatory responses (IgE, mast cells, and eosinophils), which are powerful mediators of intestinal inflammation *Overall immune response:* some evidence of acquired immunity and resistance to reinfection; immune system mechanisms stage specific; possible attachment of antibody and perhaps complement followed by cell attachment which damages cuticle, followed by phagocytosis by macrophages; increased IgE levels seen
Toxocariasis	
Visceral	Prolonged and severe T-cell-dependent eosinophilia; rapid, vigorous, prolonged humoral response; elevated IgG anti-A and anti-B blood group antigen titers present; elevated IgM anti-IgG (rheumatoid factor) titers may occur; elevated IgE titers
Ocular	May not be able to demonstrate circulating anti-*Toxocara* antibody; eosinophilic granulomas seen in some infections
Filariasis	Both tissue forms and microfilariae can partially evade the immune system, immunity eventually develops; strong immune responses against adult worms responsible not for protection but host pathology. *Overall immune response:* when parasites eliminated from blood, antibody can be found on surface of microfilariae; antibody initiates attack by various cells (IgG, IgE, eosinophils, neutrophils, like other helminth infections); IgE may be important in protection against larval stages but may also contribute to immunopathology via hypersensitivity reactions to adult worms; the T-cell anergy seen in patients heavily infected with lymphatic filariasis or with *Onchocerca* is probably due to down-regulation of Th1 responsiveness by IL-10.

[a]Data from references 1–3, 102.

The response of an infected host to a parasitic helminth involves both innate and adaptive immune factors and a host of cellular responses. A number of studies demonstrate a quantitative protective effect of Th2 immunity; however, it is unclear whether sterile immunity can be achieved. Unfortunately, helminths have the ability to modulate the host immune system, so that the host is required to overcome the parasite immunoregulation. Both IL-10 and TGF-β are closely related to regulatory T-cell activity, which is now becoming more evident in a number of human helminth infections.

The glycans of parasitic helminths have unique functions in innate immune responses and induce both C-type lectin receptor (CLR) signaling as well as cross talk with Toll-like receptor (TLR) signaling in the human system. The molecular mechanisms of glycan-dependent innate immune responses are also linked to the adaptive immune responses. Helminth infections and their products have a phenomenal ability to ameliorate responses to a variety of inflammatory disorders. For example, in clinical studies of patients suffering from inflammatory bowel disease, treatment with the pig nematode *Trichuris suis* caused remission of Crohn's disease for more than half of the patients and improved the symptoms of ulcerative colitis for many patients (91, 92). In addition to activating regulatory T cells to increase IL-10 and TGF-β, helminths act on other cells such as regulatory dendritic cells and macrophages, thus preventing the activation of effector T cells (lead to inflammation and disease). Third, worms seem to alter the bacterial composition of intestinal flora. Research in the mouse model indicates that helminths promote the growth of gut microorganisms considered to be "probiotics" that help maintain intestinal health (92). Recent studies have shown abrogation of multiple disease processes in the presence of helminth infection, including allergic airway inflammation, encephalitis, inflammatory bowel disease, rheumatoid arthritis, and type I diabetes in experimental models.

Enhanced allograft tolerance with helminth infection has been demonstrated in multiple species across multiple organ allograft models (mouse heart and skin; rat heart, liver, and kidney). These experimental data are consistent with historical results of skin grafting in established human schistosomiasis and supported by more recent suggestions of reduced immunosuppression requirement after liver transplantation for human *Echinococcus* infection. Thus, the possibility can now be entertained of including specific live (nonpathogenic) helminth infection, or defined products from immunoregulatory helminths, in future transplantation protocols (93).

Summary

Although there is evidence for acquired immunity in most protozoan infections, it is often neither fully effective nor protective. As with many organisms, the fact that the host does not die of overwhelming infections supports the concept of parasite immunity.

Since the majority of helminths do not multiply within the host, the host immune system is not faced with an overwhelming infection due to parasite multiplication as seen with protozoal infections. Many helminth life cycles include both larval and adult forms, both of which stimulate immune system reactions. However, adult and most larval forms are too large to be destroyed by antibody or by phagocytic cells. In this case, there may be some form of antibody-dependent cell-mediated cytotoxicity in which the parasites become coated with antibodies which bind eosinophils and other cells that destroy the parasites. Worm expulsion from the intestine is a CD4$^+$ T-cell-mediated event associated with type 2 cytokines. There tends to be less evidence for the implication of eosinophils in host protection. While type 1 or type 2 responses may be key factors following infection with intracellular protozoa or gut-dwelling nematodes, the mechanisms responsible for resistance to any one organism are usually quite specific for the biological characteristics of each.

General comments on a number of different parasites, both protozoan and helminth, are presented in Table 32.18. Although not all-inclusive, it is hoped that this chapter has provided some insight into the complex interactions between parasite and host.

References

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33

Antibody and Antigen Detection in Parasitic Infections

Protozoal infections
Amebiasis
Babesiosis
Chagas' disease
Cryptosporidiosis
Cyclosporiasis
Giardiasis
Leishmaniasis
Malaria
Toxoplasmosis
Trichomoniasis
Helminth infections
Cysticercosis
Hydatid disease
Fascioliasis
Filariasis
Onchocerciasis
Paragonimiasis
Schistosomiasis
Strongyloidiasis
Toxocariasis
Trichinosis
Intradermal tests
Casoni test
Montenegro test

Parasitic organisms infecting humans can be divided into two classes: (i) those that multiply within the host (e.g., protozoa) and (ii) those that mature within the host but never multiply (e.g., most helminths).

Infections caused by pathogenic protozoa, which multiply within the host, are similar to infections caused by bacterial, fungal, or viral pathogens. There is continuous antigenic stimulation of the host immune system as the infection progresses. In these instances, there is often a positive correlation between clinical symptoms and serologic test results.

Some helminths migrate through the body and pass through a number of developmental stages before becoming mature adults. With rare exceptions, these infections have been difficult to confirm serologically, possibly because of a limited antigenic response by the host or failure to use the appropriate antigen in the test system. Most antigens used in serologic procedures are heterogeneous mixtures that are not well defined. The result of using such antigens may be cross-reactions or inadequate sensitivity. Examples of test formats can be seen in Fig. 33.1 to 33.8.

Antibody Detection. Although parasites and their by-products are immunogenic for the host, the host immune response is usually not protective. Any immunity that does develop is usually species specific or even strain or stage specific. Detection of antibodies in serum or cerebrospinal fluid (CSF) can be very useful as an indicator that an individual has been infected with a specific parasite. A positive result in an individual with no exposure to the parasite prior to recent travel in a disease-endemic area may be interpreted as indicating recent infection. However, detection of specific antibodies in a person native to an area of endemicity may reflect only a past infection unrelated to current clinical status. In general, detection of antibodies to parasitic diseases indicates infection at some indeterminate time and not necessarily acute or current infection. Levels of antibodies to parasites slowly decline after the patient is cured of the infection but generally last for at least 6 months to many years, depending on the infecting parasite, and thus are not generally useful, real-time indicators of successful cure.

In certain parasitic infections, the standard diagnostic laboratory procedures may not be sufficient to confirm infection or specimen collection may not be practical or cost-effective. In these circumstances, alternative methods may be helpful;

doi:10.1128/9781555819002.ch33

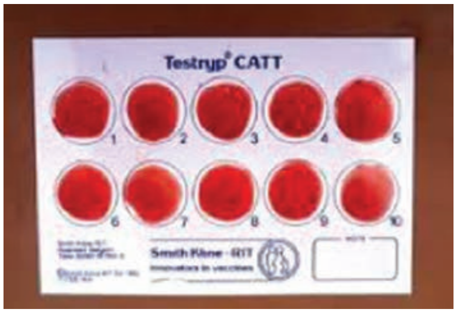


Figure 33.1 African trypanosomiasis. Card agglutination test for trypanosomiasis (CATT), antibody detection. doi:10.1128/9781555819002.ch33.f1

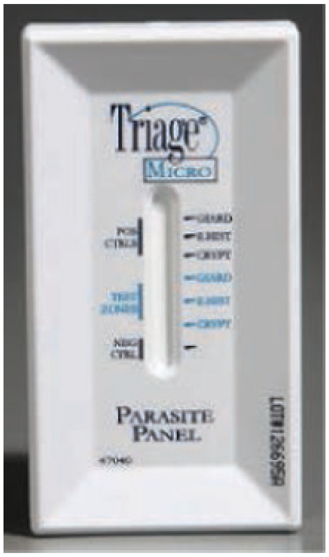


Figure 33.3 Triage rapid immunoassay. This test detects the presence of the *Entamoeba histolytica/E. dispar* group, *Giardia*, and *Cryptosporidium* spp. antigen in human fecal specimens. doi:10.1128/9781555819002.ch33.f3

these include antibody, antigen, and nucleic acid detection. In some cases, serologic methods might be clinically indicated and may be very helpful, particularly if a parasitic infection is suspected and routine results are negative. However, even with the most sophisticated technology, few serologic tests for parasitic infections can be used to confirm an infection or predict the disease outcome. Interpretation of test results may also present problems, particularly when one is dealing with patients from areas of endemic infection, who may have higher baseline titers than do patients from other areas, in whom a low titer may actually be significant. Antibody detection generally indicates exposure to the parasite at some time in the past and may not necessarily reflect a current infection. This is particularly true when testing patients who have lived in an area of endemic infection for some time; their current clinical presentation may have no relationship to a positive antibody titer for a particular parasite. Although antibody levels generally decline over a period of months to years, serologic test results neither confirm nor rule out current infection or cure.

However, a positive serologic titer to a particular parasite in a patient who has had no previous exposure to the organism is clinically relevant and probably indicates recent exposure. The importance of a complete history,

Figure 33.2 ELISA plate. Test can be used for either antibody or antigen detection; yellow color indicates a positive in this particular example. doi:10.1128/9781555819002.ch33.f2

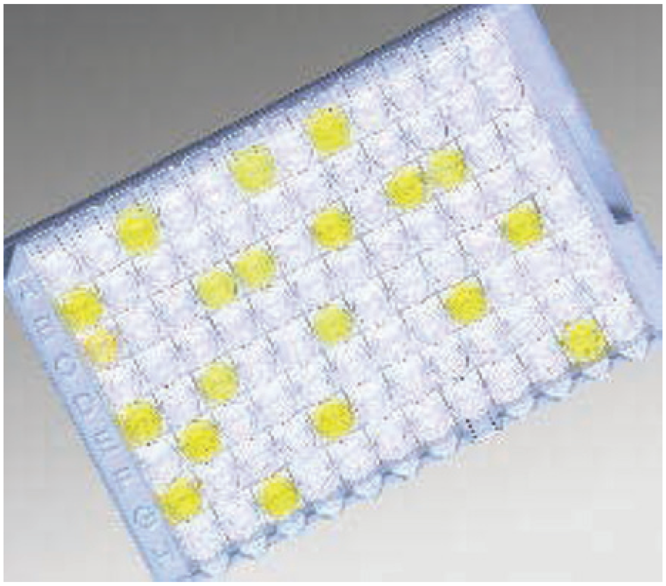


Figure 33.4 Rapid immunoassay for *Cryptosporidium* spp. This rapid cartridge format detects the presence of *Cryptosporidium* spp. antigen in human fecal specimens. The cartridge at upper left demonstrates the control spot, while the lower indicates the test is positive (second spot). doi:10.1128/9781555819002.ch33.f4

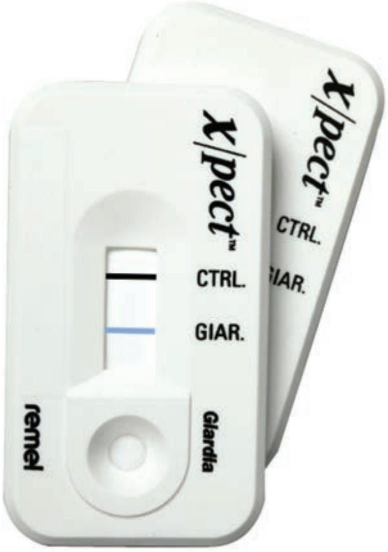
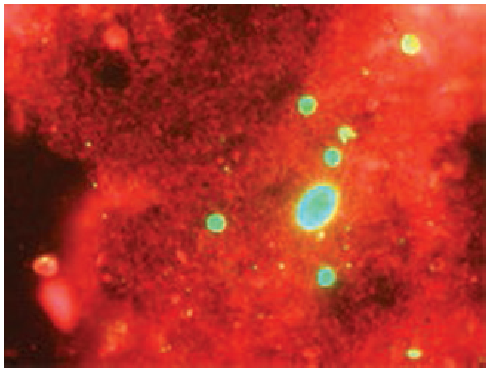


Figure 33.5 Rapid immunoassay for *Giardia*. This rapid cartridge format detects the presence of *Giardia* antigen in human fecal specimens. The cartridge shows a black control line and a blue test line, indicating a positive result.
doi:10.1128/9781555819002.ch33.f5

including both residence and travel information, is critical for accurate interpretation of serologic test results. Specific reagents available for serologic diagnostic testing are listed in Tables 33.1 and 33.2.

Immunodiagnostic testing for parasitic diseases is complicated by the general assumption that serology tests for antibodies to a disease will detect all infections caused by any of the species that infect humans. That assumption is not true; some serologic tests, such as those for schistosomiasis, detect only species-specific antibodies. The same specimen can have a negative result in one test and a positive result in another.

Figure 33.6 Example of the *Giardia*/*Cryptosporidium* combination FA test result (with test [yellow-green] and counterstain [orange] colors); the large object is a *Giardia* cyst, while the smaller objects are *Cryptosporidium* spp. oocysts.
doi:10.1128/9781555819002.ch33.f6



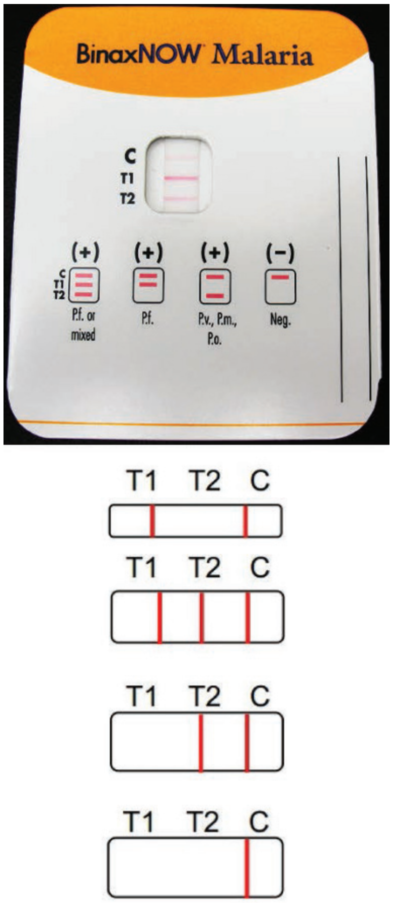


Figure 33.7 *Plasmodium* spp. rapid test (membrane flow): BinaxNOW card (upper) and the four possible results (lower). Interpretation of the four examples of results: (top set of lines) the presence of colored lines at T1 and C indicates a positive result for an infection with *P. falciparum*; (second set) the presence of three colored lines (T1, T2, and C) indicates a positive result for *P. falciparum* monoinfection or mixed infection among the four kinds of plasmodium; (third set) the presence of colored lines at T2 and C indicates a negative result for *P. falciparum* and a positive result for non-*P. falciparum* (*P. vivax*, *P. malariae*, or *P.ovale*) monoinfection or mixed infection; (bottom set) the presence of one line at C indicates a negative result. This card was not designed to detect *P. knowlesi*; however, modifications are under way. doi:10.1128/9781555819002.ch33.f7

Although serologic test procedures for parasite antibodies have been available for many years, they are not routinely offered by most clinical laboratories for the reasons mentioned above (sensitivity, specificity, and interpretation). Standard techniques that have been used include complement fixation (CF), indirect hemagglutination (IHA), indirect fluorescent antibody (IFA), enzyme immunoassay (EIA), enzyme-linked

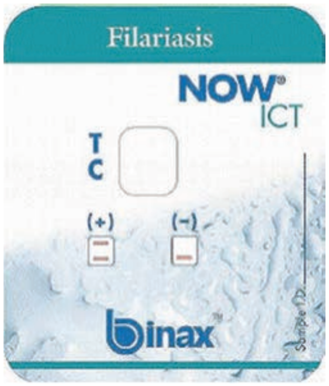


Figure 33.8 Filariasis rapid test (membrane flow). The test is also specific, as it tests for specific antigens for lymphatic filariasis and allows for the differentiation between microfilarial diseases. The most useful antigen detection test is the antigen card test known as the ICT. Not only is this test highly specific and highly sensitive, but it also quick (ready in 10 minutes), mobile, and cheap. In addition, this test can be administered during the day or night, unlike other tests that require blood samples to be taken when microfilariae are at high levels in the blood. The antigen detection test cannot differentiate between active and inactive microfilariae. Antigens can still be present in the blood if any trace of dead microfilariae remains in the circulation. Hence, a positive ICT test result may not mean the patient has active, living microfilariae in the blood. Note that if only the control line is positive, this indicates a negative result; both lines positive indicates a positive result. doi:10.1128/9781555819002.ch33.f8

immunosorbent assay (ELISA), immunoblot, soluble-antigen fluorescent antibody, bentonite flocculation, latex agglutination (LA), double diffusion, counterimmunoelectrophoresis (CIE), immunoelectrophoresis, radioimmunoassay, and intradermal tests (Table 33.3). Excellent reviews of these procedures, the rationale for their use, and their advantages and limitations are provided in references 1 and 2. Immunodiagnostic tests for amebiasis, cryptosporidiosis, filariasis, giardiasis, malaria, cysticercosis, schistosomiasis, and African trypanosomiasis have very limited commercially or U.S. Food and Drug Administration (FDA)-approved antibody detection tests available. Experimental results have been too variable due to the type of antigen preparations used (e.g., crude, recombinant purified, adult worm, egg) and also because of the use of nonstandardized test procedures. Cross-reaction leading to false-positives and misdiagnosis is also a problem, especially in regions where more than one parasite is endemic.

The Centers for Disease Control and Prevention (CDC) offers a number of serologic test procedures for diagnostic purposes, some of which are not available elsewhere. Because regulations about submission of specimens may vary from state to state, each laboratory should check with its own county or state department of public health for the appropriate instructions. Additional information on procedures, availability of skin test antigens, and interpretation of test results may be obtained directly from CDC by writing or calling:

Serology Unit
Parasitology Diseases Branch
Centers for Disease Control and Prevention
Building 4, Room 1009, Mail Stop F13
4770 Buford Highway
Atlanta, GA 30034
Serology: (770) 488-7760
Chagas' disease and leishmaniasis: (770) 488-4474
Malaria: (770) 488-7765.

General guidelines for specimen collection can be seen in Table 33.4.

Antigen Detection. For most parasitic diseases, the antigen(s) is generally the assay component which has the most influence on test sensitivity and specificity. Parasites generally have more than one life cycle stage that may have both mutually shared antigens and stage-specific antigens. The matrix to which antigens are bound for use in a specific procedure also influences which antigen subset will be available for antibody binding. During the past few years, changes in the availability of certain tests have occurred, both at the CDC and at some private laboratories. The currently available antibody and antigen detection tests are listed in Table 33.1. A number of antibody and/or antigen detection tests have been developed in the research laboratory but are not yet available commercially; these include tests for *Ancylostoma caninum*, *Angiostrongylus*, *Anisakis*, *Baylisascaris*, *Gnathostoma*, microsporidia, *Opisthorchis*, and *Trypanosoma brucei* (2). Although PCR tests have been developed for most human parasites, they are generally available only through various reference or research laboratories. As the costs of these antigen detection tests decrease and automation of the various steps is developed, there will be more interest in incorporating these methods into the routine diagnostic laboratory. A number of rapid methods are currently available for protozoa found in the intestinal tract. However, in spite of their high levels of sensitivity and specificity, there are other pathogens for which these tests are not available, including *Blastocystis* spp., *Dientamoeba fragilis*, and the microsporidia.

Molecular Methods. Progress has been made in the development and application of molecular methods for antigen detection, including the use of purified or recombinant antigens and nucleic acid probes. The detection of parasite-specific antigen is more indicative of current disease. Many of the assays were originally developed with polyclonal antibodies which were targeted to unpurified antigens that markedly decreased the sensitivity and specificity of the tests. Immunoassays are generally simple to perform and allow a large

TABLE 33.1 Serologic, antigen, and probe tests used in the diagnosis of parasitic infections[a]

Disease	Routine antibody test(s)	Antigen, probe test(s), PCR, NAT
Protozoa		
Amebiasis (*Entamoeba histolytica*)	EIA	EIA, Rapid, multiplex PCR
Babesiosis	IFA (CDC)	
Chagas' disease	EIA, IFA (CDC)	
Cryptosporidiosis		DFA, EIA, IFA, Rapid, multiplex NAT, multiplex PCR
Entamoeba histolytica/E. dispar		EIA, Rapid
Giardiasis		DFA, EIA, Rapid, multiplex NAT, multiplex PCR
Cryptosporidium/Giardia combination		DFA, IFA, EIA, Rapid, multiplex PCR
Cryptosporidium, *Giardia*, *Entamoeba histolytica/E. dispar* combination		Rapid
Cyclosporiasis		Multiplex PCR
Leishmaniasis	IFA (CDC), EIA	PCR
	(Visceral/EIA) (PDC)	PCR (Walter Reed Army Medical Center)
Malaria	IFA (4 species) IFA (PDC)	Rapid, PCR (CDC) PCR (PDC)
Microsporidiosis		IFA[b]
Toxoplasmosis	EIA IgG, EIA IgM Immunocapture, ISAGA IgM, IFA, LA	PCR
Trichomoniasis		DFA, LA, DNA probe
Trypanosomiasis (African sleeping sickness)	CATT	PCR
Trypanosomiasis (Chagas' disease)	EIA, IFA (CDC), EIA	PCR
		PCR (PDC)
Helminths		
Angiostrongyliasis	EIA (PDC)	
Ascariasis	EIA (PDC)	
Baylisascariasis	IB (serum/CSF)	
Cysticercosis	EIA, IB (serum/CSF) (CDC) Serum, CSF (PDC)	
Echinococcosis	EIA, IB EIA (PDC)	
Fascioliasis	EIA, IB EIA (PDC)	
Filariasis	EIA (CDC)	Rapid, EIA
	EIA (PDC)	
Gnathostomiasis	IB (Mahidol University, Thailand)	
Hookworm	EIA (PDC)	
Paragonimiasis	EIA, IB, WB (CDC)	
	EIA (PDC)	
Schistosomiasis	EIA, IB, FAST-ELISA (CDC)	EIA
	EIA (PDC)	
Strongyloidiasis	EIA (CDC)	
	IgG4 (PDC)	
Toxocariasis	EIA (CDC)	
	EIA (PDC)	
Trichinosis	BF, EIA (CDC) EIA (PDC)	

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[a] BF, bentonite flocculation; CATT, card agglutination test for trypanosomiasis; CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; EIA, enzyme immunoassay; FAST-ELISA, Falcon assay screening test-enzyme-linked immunosorbent assay; IB, immunoblot; IFA, indirect fluorescent antibody; ISAGA, immunosorbent agglutination assay; LA, latex agglutination; NAT, nucleic acid test; PDC, Parasitic Disease Consultants (Dr. Irving G. Kagan); Rapid, rapid immunochromatography (some are in cartridge formats); WB, Western blot.

[b] Reagents are not commercially available within the United States but have been developed (for microsporidia).

TABLE 33.2 Antibody detection kits available commercially within the United States for the diagnosis of parasitic diseases other than toxoplasmosis[a]

Disease (parasite)	Company[b]	Antibody test format[c]
Protozoa		
Amebiasis (*Entamoeba histolytica*)	Chemicon	EIA
	R-Biopharm	EIA
	IVD Research	EIA
Chagas' disease (*Trypanosoma cruzi*)	Chemicon	EIA
	Hemagen Diagnostics	EIA
	IVD Research	EIA
	InBios	EIA, Rapid
Leishmaniasis, visceral (*Leishmania* spp.)	Immunetics	IB
	R-Biopharm	EIA
	InBios	Rapid
	IVD Research	EIA
Helminths		
Cysticercosis (*Taenia solium*)	Chemicon	EIA
	R-Biopharm	EIA
	Immunetics	IB
	IVD Research	EIA
	InBios	EIA
Echinococcosis (*Echinococcus granulosus*)	Immunetics	IB
	R-Biopharm	EIA
	IVD Research	EIA
Echinococcosis (*E. multilocularis*)	Bordier Affinity	EIA
Schistosomiasis (*Schistosoma* spp.)	IVD Research	EIA
Strongyloidiasis (*Strongyloides stercoralis*)	IVD Research	EIA
Toxocariasis (*Toxocara canis*)	IVD Research	EIA
	R-Biopharm	EIA
Trichinosis (*Trichinella spiralis*)	IVD Research	EIA
	R-Biopharm	EIA

[a] Data from CDC.

[b] Bordier Affinity Products, Chatanerie 2, CH-1023, Crissier, Switzerland; Chemicon, 28835 Single Oak Dr., Temecula, CA 92950; Hemagen Diagnostics, 34–40 Bear Hill Rd., Waltham, MA 02154; Immunetics, 380 Green St., Cambridge, MA 02139; InBios, 562 1st Avenue South, Suite 600, Seattle, WA 98104; IVD Research, 5909 Sea Lion Place, Suite D, Carlsbad, CA 92008; R-Biopharm AG, An der Neuen Bergstrasse 17, 64297 Darmstadt, Germany.

[c] IB, immunoblot; Rapid, rapid immunochromatographic.

number of tests to be performed at one time, thereby reducing overall costs. A major disadvantage of detection of antigens in stool specimens is that the method can detect only one or two pathogens at a time. One still must perform a routine ova and parasite examination to detect other parasitic pathogens. The current commercially available antigen tests have excellent sensitivity and specificity compared with routine microscopy (Tables 33.1 and 33.5).

There are several FDA-approved molecular diagnostic tests for parasites. They include the APTIMA *Trichomonas vaginalis* Assay (GenProbe, San Diego, CA). This assay utilizes the same amplification technology that has been used for years to detect *Neisseria gonorrheae* and *Chlamydia trachomatis*. It also uses the same specimen types (i.e., clinician-collected vaginal and endocervical swabs, female urine, and PreservCyt solution). It has reported high sensitivity and specificity, and does not require organism viability or motility. It, as well as other antigen-based assays, represents advances in the detection of this parasite.

The Affirm VPIII DNA probe technology (Becton Dickinson, Franklin Lakes, NJ) offers a dependable, rapid means for the early identification of three organisms causing vaginitis: *Candida* species, *Gardnerella vaginalis*, and *Trichomonas vaginalis*. During the test protocol, the assay aligns complementary nucleic acid strands to form specific, double-stranded complexes called hybrids. For each organism, this hybrid is composed of capture and color development single-stranded nucleic acid probes, complementary to the released target nucleic acid analyte. Enzyme conjugate then binds to the captured analyte.

In January 2013, the FDA approved the first test that can simultaneously detect 11 common viral, bacterial, and

TABLE 33.3 Serology-based assays: examples of various methods

Assay	Description
IHA	The indirect hemagglutination (hemagglutination inhibition) assay detects the presence of specific antiparasitic antibodies in a test serum. The serum, usually as a series of dilutions, is prepared prior to the addition of a standard suspension of erythrocytes. The highest dilution of serum that inhibits hemagglutination is the HI titer of the serum.
DFA	Primary, or direct, immunofluorescence uses a single, primary antibody, chemically linked to a fluorophore. The primary antibody recognizes the target molecule (antigen) and binds to a specific region called the epitope. The attached fluorophore can be detected via fluorescent microscopy, which, depending on the messenger used, will emit a specific wavelength of light once excited. Direct immunofluorescence, although somewhat less common, has notable advantages over the secondary (indirect) procedure. This approach has been used for the detection of antibodies to *Entamoeba histolytica*.
IFA	Indirect immunofluorescence uses two antibodies: the unlabeled first (primary) antibody specifically binds the target molecule, and the secondary antibody, which carries the fluorophore, recognizes the primary antibody and binds to it. Multiple secondary antibodies can bind a single primary antibody. This provides signal amplification by increasing the number of fluorophore molecules per antigen. This protocol is more complex and time-consuming than the primary (or direct) protocol above, but it allows more flexibility because a variety of different secondary antibodies and detection techniques can be used for a given primary antibody.
ELISA	The enzyme-linked immunosorbent assay can be performed to evaluate either the presence of antigen or the presence of antibody in a sample. Relevant antigens are attached to a surface (usually 48- to 96-well trays). Then, a serum antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate. It is a useful tool for determining serum antibody concentrations for certain parasitic infections, such as Chagas' disease, leishmaniasis, toxoplasmosis, and some of the helminth infections.
FAST-ELISA	The Falcon assay screening test ELISA uses synthetic and recombinant peptides to evaluate antibody responses to an antigen. In the past, the method has been applied to the study of malaria, fascioliasis, schistosomiasis, and taeniasis. However, this technique is subjected to the same drawbacks as most serology-based tests. Antibodies raised against a peptide from one parasite protein may cross-react with proteins from other species. Also, antibodies against a peptide may react in some assays but not in others and some regions of a peptide may be more immunogenic than others.
Dot-ELISA	The main difference between the regular ELISA and the dot-ELISA is in the surface used to bind the antigen. In the dot-ELISA, the plastic plate is replaced by a nitrocellulose or other paper membrane onto which a small amount of sample volume is applied. The change in binding matrix improves the specificity and sensitivity of the assay by reducing the binding of nonspecific proteins when plastic binding matrixes are used. The principle is similar to the immunoblot. The dotted membrane is incubated first with an antigen-specific antibody followed by an enzyme-conjugated anti-antibody. The addition of a precipitable, chromogenic substrate causes the formation of a colored dot on the membrane which can be visually read. The dot-ELISA has been and still is extensively used in the detection of parasitic diseases, including amebiasis, babesiosis, fascioliasis, cutaneous and visceral leishmaniasis, cysticercosis, echinococcosis, malaria, schistosomiasis, toxocariasis, toxoplasmosis, trichinosis, and trypanosomiasis. In the last few years, published studies have demonstrated the use of the dot-ELISA for the detection of *Fasciola gigantica*, *Trypanosoma cruzi*, and *Trypanosoma brucei*.
LIPS	The luciferase immunoprecipitation system is a modified ELISA-based assay; serum containing antigen-specific antibodies can be identified by measuring light production. An antigen is fused to the enzyme reporter Renilla luciferase (Ruc) and expressed as a Ruc-fusion in mammalian cells to allow for mammalian-specific posttranslational modifications. The crude protein extract is then incubated with the test serum and protein A/G beads. During the incubation, the Ruc-antigen fusion becomes immobilized on the A/G beads, which allows the antigen-specific antibody to be quantitated by washing the beads and adding coelenterazine substrate and measuring light production. LIPS has been used for the identification of serum samples containing *Strongyloides stercoralis* and *Loa loa* antibodies. Some of the advantages of the LIPS technology include its rapidity and accuracy in detecting infected patients.
IB	When the immunoblot (Western blot) is used, a specific protein is transferred to a nitrocellulose membrane, nonspecific sites are blocked, and the protein is probed with serum samples. In the case of testing for *Baylisascaris* antibodies, the serum was diluted at 1:100. For high-throughput testing of sera, 6 µg of rBpRAG1 protein was separated on NuPage one-dimensional-well 4–12% Bis-Tris gradient gels (Life Technologies) and transferred to nitrocellulose membranes for Western blot analysis by using a Mini-Protean II multiscreen apparatus (Bio-Rad Laboratories). Human serum and CSF specimens were tested at dilutions of 1:100 and were incubated with HRP-conjugated anti-human IgG at 1:5,000. The membranes were incubated with DAB-H2O2 solution. NOTE: The lack of cross-reactivity in the rBpRAG1 assay eliminates the need for additional testing to rule out toxocariasis and increases the confidence in a baylisascariasis diagnosis.[a]

[a] Adapted from reference 101.

TABLE 33.4 Specimen collection and shipment for antibody and antigen detection tests

Type of test requested	Collection	Shipment
Antibody detection		
Most parasites	Serum or plasma acceptable	All specimens can be shipped at room temperature to arrive within several days; if specimen is small (<1.0 ml), shipment with dry ice is recommended to prevent specimen evaporating during shipment; in general, acute- and convalescent-phase sera are not required; not all laboratories are equipped to perform testing on eye fluids or CSF; advisable to call to confirm availability if other than CDC
Toxocariasis, toxoplasmosis	Aqueous and vitreous eye fluids accompanied by serum	
Central nervous system infections (cysticercosis, toxoplasmosis)	CSF accompanied by serum	
Antigen detection		
Fresh or formalinized stool	Routine stool collection vials	Some diagnostic kits require fresh stool; advisable to check with the laboratory prior to specimen submission

parasitic (*Cryptosporidium*, *Giardia*) causes of infectious gastroenteritis from a single patient sample (xTAG Gastrointestinal Pathogen Panel; Luminex, Inc., Austin, TX). The xTAG GPP is a multiplexed nucleic acid test that detects various causes of gastroenteritis, inflammation of the stomach, and intestines.

The BioFire FilmArray Gastrointestinal Panel is another FDA-approved multiplex PCR test that can simultaneously detect 22 gastrointestinal pathogens in about an hour (BioFire, Salt Lake City, UT). The panel includes *Cryptosporidium* sp., *Cyclospora cayetanensis*, *Entamoeba histolytica*, and *Giardia lamblia*, along with a number of bacteria and viruses.

There are also a number of other molecular tests that are in clinical trials for the detection of select gastrointestinal parasites. These tests are molecular gastrointestinal panels and target the most commonly occurring bacterial, viral, and parasitic stool pathogens. Although there are laboratory-developed tests for most parasites, these are not commercially available or available only in specialized testing centers. When such tests are used, there should be attention given to the use of internal amplification controls to detect inhibition, since common specimens, such as blood and stool, may contain PCR inhibitors. Thorough validation is required before these are implemented for clinical testing.

Protozoal Infections

Amebiasis

The causative agent of amebiasis, *Entamoeba histolytica*, is found throughout the world and is a major infectious disease in developing countries. Although this parasite is considered mainly a cause of diarrhea, the trophozoite stage can readily invade tissues, resulting in the development of extraintestinal disease.

The diagnosis of infection with *E. histolytica*/*E. dispar* is usually based on finding either trophozoite or cyst stages in the stool. However, unless the trophozoites contain ingested red blood cells (RBCs), morphologic differentiation between pathogenic *E. histolytica* and nonpathogenic *E. dispar* is not possible. Antigen detection may be helpful as an adjunct to routine microscopy, either for detection of the *E. histolytica*/*E. dispar* group or for detection of pathogenic *E. histolytica*. In extraintestinal amebiasis, one may or may not find organisms in the stool. The diagnosis of extraintestinal amebiasis depends largely on serologic tests for antibody detection.

The discovery that there are two distinct but morphologically identical species of *Entamoeba* infecting humans is important. *E. dispar* is associated with the asymptomatic carrier state and is the most prevalent species, whereas *E. histolytica* is the pathogenic form capable of invading tissue and causing symptomatic disease. Through the use of RNA and DNA probes, the genetic difference between these two species has been substantiated. At present, when a form resembling *E. histolytica* is detected in fecal material, it is not possible to differentiate it from *E. dispar* unless the trophozoite contains ingested RBCs.

Antibody Detection. If IgG antibodies specific to *E. histolytica* are detected, tissue invasion has occurred and may represent past or present disease. Serum antibodies can be detected in 85 to 95% or more of the patients with invasive disease. In newborns, maternal antibody disappears within 3 months, and persistence of antibody after this time may indicate active extraintestinal disease.

The most commonly used serologic tests are IHA, IFA, CF, ELISA, LA, CIE, and immunodiffusion (ID). CF tests have not been widely used because of nonspecific and variable results. IHA and LA appear to detect the same antibody. Although ELISA and IHA are two of the more sensitive tests, antibody titers persist for years, making interpretation difficult in certain patients. ELISA and IHA are useful for epidemiologic surveys. IFA and ID not only are sensitive tests but also have high positive predictive values. After successful therapy, titers in many of the patients decrease, allowing physicians to monitor the therapeutic response. Although tests for the detection of IgM antibodies specific

TABLE 33.5 Antigen detection kits available commercially within the United States for the immunodetection of parasitic organisms or antigens[a]

Organism and kit name	Company[b]	Test format[c]
Entamoeba histolytica		
E. histolytica II	TechLab	Rapid
ProSpecT Entamoeba histolytica	Remel	EIA
Giardia lamblia (*duodenalis*, *intestinalis*)		
Giardia Fecal Antigen Detection Lateral Flow	IVD Research	Rapid
Xpect Giardia	Remel	Rapid
ProSpecT Giardia, ProSpecT Giardia EZ	Remel	EIA
Cryptosporidium spp.		
Cryptosporidium	IVD Research	EIA
PARA-TECT Cryptosporidium	Medical Chemical	EIA
Cryptosporidium	Novocastra	DFA
ProSpecT Cryptosporidium	Remel	EIA
Xpect Cryptosporidium	Remel	Rapid
Cryptosporidium II Giardia/Cryptosporidium Quik Chek	TechLab–Inverness Medical Professional Diagnostics (formerly Wampole)	EIA
Cryptosporidium spp. and *Giardia lamblia*		
ColorPAC	Becton Dickinson	Rapid
Crypto/Giardia	IVD Research	DFA, EIA
PARA-TECT Cryptosporidium/Giardia	Medical Chemical	DFA
Merifluor	Meridian Bioscience	DFA
ImmunoCardSTAT	Meridian Bioscience	Rapid
ProSpecTGiardia/Cryptosporidium	Remel	EIA
Xpect Giardia/Cryptosporidium	Remel	Rapid
Giardia/Cryptosporidium CHEK	TechLab–Inverness Medical Professional Diagnostics (formerly Wampole)	EIA
Cryptosporidium spp., *Giardia lamblia*, and *Entamoeba histolytica*		
Triage	BioSite	Rapid
Entamoeba histolytica		
E. histolytica/E. dispar	IVD Research	EIA
ProSpecT	Remel	EIA
Entamoeba histolytica II	TechLab–Inverness Medical Professional Diagnostics (formerly Wampole)	EIA
Giardia lamblia		
PARA-TECH Giardia	Medical Chemical	EIA
Giardia	Novocastra	DFA
ProSpecT	Remel	EIA
Xpect	Remel	Rapid
Giardia II	TechLab–Inverness Medical Professional Diagnostics (formerly Wampole)	EIA
Trichomonas vaginalis		
Affirm VPIII	Becton Dickinson	Probe
T. vaginalis	Chemicon	DFA
OSOM Trichomonas	Genzyme	Rapid
XenoStrip-Tv	Xenotope	Rapid

[a] Adapted from CDC.

[b] Becton Dickinson Diagnostic Systems, 7 Loveton Cir., Sparks, MD 21152; BioSite, 11030 Roselle St., San Diego, CA 92121; Chemicon, 28835 Single Oak Dr., Temecula, CA 92590; Genzyme Diagnostics, One Kendall Square, Cambridge, MA 02139; Inverness Medical Professional Diagnostics (formerly Wampole), P.O. Box 1001, Cranbury, NJ 08512; IVD Research, 5909 Sea Lion Place, Suite D, Carlsbad, CA 92008; Medical Chemical Corp., 19430 Van Ness Ave., Torrance, CA 90501; Meridian Bioscience, 3471 River Hills Dr., Cincinnati, OH 45244; Novocastra, 30 Ingold Rd., Burlingame, CA 94010; Remel, P.O. Box 14428, Lenexa, KS 66215; TechLab, VPI Research Park, 1861 Pratt Dr., Blacksburg, VA 24060; Xenotope Diagnostics, 3463 Magic Dr., Suite 350, San Antonio, TX 78229.

[c] DFA, direct fluorescent antibody; EIA, enzyme immunoassay; IFA, indirect fluorescent antibody; Rapid, rapid immunochromatographic.

for *E. histolytica* have been reported, their clinical usefulness is questionable because of the lack of sensitivity. Many of the current tests use crude trophozoite extracts, whereas a few use recombinant proteins (3).

IHA has for many years been the standard for the diagnosis of extraintestinal amebiasis; titers of ≥1:256 are seen in 95% of patients with extraintestinal amebiasis, 70% of patients with active intestinal disease, and 10% of asymptomatic cyst passers. Unfortunately, titers can persist for many years after successful therapy, and so the presence of a titer does not automatically indicate active disease. However, false-positive results at titers of >1:256 rarely occur. The sensitivity of detection of specific antibodies to *E. histolytica* in serum is reported to be near 100%, which is promising for diagnosis of amebic liver abscess (ALA) (4). Serum anti-lectin IgG antibodies could be present within 1 week after the onset of symptoms in patients with amebic colitis and ALA, with a value over 95%.

Recombinant proteins such as the 170-kDa subunit of the galactose-inhibitable adherence lectin have been used to detect serum antibodies in more than 95% of patients with ALA. PCR and DNA hybridization assays have been used to detect specific gene sequences related to pathogenic *E. histolytica;* however, none are commercially available at this time.

In most laboratories, EIA (ELISA) has replaced other test formats; the EIA is equally sensitive and specific. The sensitivity of the EIA varies with the disease process such that 90 to 100% of individuals with extraintestinal amebiasis are positive while only 70 to 80% of those with invasive disease limited to the intestine are positive. Significant antibody titers may take 10 to 14 days to develop even with documented invasive amebiasis. Since positive titers may persist for many years after infection, a single test result must be interpreted with caution when prior infection is possible.

Antigen Detection. Antigen-based fecal immunoassays have several significant advantages over other methods currently used for diagnosis of amebiasis: (i) some of the assays differentiate *E. histolytica* from *E. dispar*; (ii) they have excellent sensitivity and specificity; (iii) they are readily usable by most laboratory personnel; and (iv) these assays have potential use as screening tools in situations such as waterborne outbreaks. Because there are distinct genetic differences between *E. dispar* and *E. histolytica*, commercial kits have been developed to detect their presence and differentiate them in clinical samples (Table 33.5). However, current antigen detection tests require the examination of fresh or frozen (not preserved) stool specimens, while many laboratories have switched to stool collection methods using various preservatives.

Other specimens in which amebic antigens have been detected include saliva, serum, and abscess fluid. With ELISA, the adherence lectin antigen has been detected in saliva samples of ALA patients. This assay was found to be 22% sensitive and 97.4% specific. Amebic antigen in pus specimens from ALA patients was detected by ELISA (4).

A coagglutination test for the detection of circulating antigen in sera from patients with ALA has been reported (5). This is a simple and economical slide agglutination test. In patients with ALA, 45 (90%) of 50 sera were positive by the coagglutination test, while none of the 25 control sera was positive. However, two false-positive results were seen with two sera from patients with other parasitic and miscellaneous infection controls. Although this test is not yet commercially available, it could be used as a sensitive and specific rapid slide agglutination test for the presence of amebic antigen in the sera; it would also be useful in a routine clinical laboratory setting. Several of the new automated enteric panels include testing for amebiasis (*Entamoeba histolytica*); additional information can be found in Appendix 8.

Babesiosis

Definitive diagnosis of babesiosis may be difficult because of the small number of infected RBCs, or the inability to discriminate between *Plasmodium falciparum* and *Babesia* spp. Babesiosis is transmitted by ticks, which can also transmit *Borrelia burgdorferi* and *Ehrlichia* spp. There are reports of coinfection with these three disease-causing agents (6). Although *Babesia microti* is the primary cause of human babesiosis in the United States, at least four other species have been associated with human infections including *Babesia duncani*, *Babesia* sp. CA-type, *Babesia* sp. MO1, and newly discovered *Babesia* sp. from a patient in Tennessee (7). Four severe to fatal human cases with *Babesia* sp. CA-type have been reported in splenectomized patients from California (8). This species is morphologically indistinguishable from *B. duncani*, but can be distinguished by genetic sequencing of multiple targets. In the United States, serologic tests for *B. microti* are considered sensitive and specific because the other *Babesia* that infect humans in the United States (i.e., *B. duncani*, *Babesia* sp. CA-type, and *Babesia* sp. MO1) have limited reactivity with *B. microti* antigens. But, as new *Babesia* species are discovered, serologic cross-reactivity may be observed.

The serologic response to acute infection is marked by a rise in the titer to ≥1:1,024 during the first few weeks, followed by a gradual decline to 1:16 to 1:256 over a period of 6 months. Infections caused by a strain of *Babesia* found in the western part of the United States may not yield a diagnostic titer (9) and would represent a false-negative result. When tested for babesiosis by IFA, patients infected with malaria have been noted to yield a serologic cross-reaction, generally of low titer; however, the test is 100% specific for patients with other tick-borne infections or patients with no exposure to the organism. Titers of ≥1:64 indicate infection with *B. microti*. Titers may persist for 6 to 12 months after acute illness. A titer range of 1:64 to 1:256 suggests an infection probably more than 6 months ago. However, a titer of ≥1,024 indicates a relatively recent infection.

PCR has also been used to detect infections (10). Despite the present unavailability of commercially available screening assays, some form of serologic and nucleic acid testing for *Babesia* spp. may be justified. Assuming that interactions between humans and ticks will probably increase in the future, it is likely that both new and extant tick-borne agents pose potential threats to transfusion safety (11).

Chagas' Disease

Trypanosoma cruzi infects millions of people in Central and South America and is a significant public health problem there. Screening of blood donors in areas where Chagas' disease is endemic is very important. In addition, donor centers are concerned about contamination of the blood supply when donors are migrant workers or immigrants who resided in areas where infection is endemic. Definitive diagnosis depends on demonstration of the parasite in blood or tissues. However, examination of blood may be helpful only during the initial acute disease or during chronic exacerbation. During chronic Chagas' disease, serologic testing may be the only method of detecting the infection.

Antibody Testing. Serologic tests used for the diagnosis of Chagas' disease include CF (Guerreiro-Machado test), IFA, IHA, ELISA, and agglutination. Cross-reactions with low titers have been noted with leishmaniasis, infectious mononucleosis, lepromatous leprosy, pemphigus foliaceus, and *Trypanosoma rangeli* infections.

Although the IFA test is more sensitive than the CF test, the CF test is more specific. ELISA kits which use crude antigens have sensitivities and specificities similar to those of IFA. For chronic trypanosomiasis, sensitivity is good and negative serology is helpful to exclude the diagnosis. In acute cases of *T. cruzi*, the sensitivity is lower because it takes from 2 to 8 weeks to develop a detectable antibody titer. The specificity of the test is reasonable but cross-reactions with leishmaniasis and syphilis were observed.

Use of synthetic peptides and recombinant antigens has improved the sensitivity and specificity of these diagnostic techniques (12, 13). Tri- and tetrapeptides, representing immunodominant epitopes of *T. cruzi*, have been used in the ELISA format to detect serum antibodies. These tests showed much higher rates of sensitivity and specificity and may serve as excellent alternatives to lysate for detection of anti-*T. cruzi* antibodies, as required for developing blood-screening assays (14, 15). Recombinant antigen-based assays have been used for the detection of IgM and IgA antibodies in congenital Chagas' disease and for the evaluation of therapy (16).

In one study to determine the prevalence of Chagas' disease among donors in five Mexican blood banks, two commercial serologic assays for Chagas' disease, the Abbott Chagas EIA and the Meridian Chagas' IgG ELISA, were tested. It is obvious from the data that transfusion-associated transmission of *T. cruzi* is occurring in the study areas. Serologic testing of blood donors for Chagas' disease should be performed there and in the rest of Mexico. However, the two screening assays evaluated may lack the accuracy necessary for blood donor testing when used as suggested by the manufacturers (17).

Routine blood donor screening for *T. cruzi* with a single test results in unacceptable numbers of false-negative samples in areas of highly endemic infection or in at-risk population groups. Adding a second test seems mandatory, but selection depends on local costs and feasibility (18). Because of the risk of transfusion-related infections and the lack of adequate screening tests to detect *T. cruzi*-positive donors, PCR may become the test of choice for screening (19).

Radioimmunoprecipitation assay (RIPA) has been used in the United States as the "gold standard." Although these tests are sensitive and specific, there is a need for a rapid, sensitive, and specific diagnostic test for screening surveys or use in small rural clinics or in cardiac transplantation situations. Such a test needs to maintain a high level of sensitivity and specificity irrespective of geographical location. Using recombinant proteins, a lateral flow assay was performed with the ITC6 recombinants as the solid-phase antigen in the test line. An affinity-purified goat anti-human IgG antibody was labeled with colloidal gold and used as the mobile phase. The control line comprised recombinant protein A. The ITC8.2 assay was shown to have a high correlation with the confirmatory RIPA used in the United States and demonstrates improved reactivity with *T. cruzi*-positive sera from Central American and U.S. blood donors compared to TcF (20).

There are currently two FDA-approved assays for the detection of *T. cruzi* antibodies. The ABBOTT PRISM Chagas EIA (Abbott Laboratories, Abbott Park, IL) is intended to screen individual human donors, including volunteer donors of whole blood and blood components and other living donors, for the presence of antibodies to *T. cruzi*. The assay is also intended for use in testing serum and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens to screen cadaveric (non-heart-beating) donors. In a study of sensitivity and specificity in a high-risk population, the ABBOTT PRISM Chagas assay demonstrated a 98.47% sensitivity and a specificity of 98.72%.

The ORTHO T. cruzi ELISA (Ortho-Clinical Diagnostics, Inc., Raritan, NJ) is for the qualitative detection of antibodies to *T. cruzi* in human serum, plasma, and cadaveric specimens, similar to the Abbott product. The ORTHO T. cruzi ELISA Test System is intended for use in a fully manual mode, in semi-automated mode using the Ortho Summit Sample Handling System (Summit), or in automated mode with the Ortho Summit System. Sensitivity was 99.99% from normal blood donors and 100% when tested from countries with endemic *T. cruzi*. Specificity from the high-risk population was 99%. These products are not approved for pooled blood or processed plasma and products made from such pools (FDA: http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/UCM080466#anti_Tcruzi_Assays, accessed 19 June 2014).

Antigen Testing. Circulating-antigen detection methods have been used to detect recently acquired and congenital infections. PCR has greater sensitivity and specificity than routine methods for the diagnosis of Chagas' disease and can detect as few as one trypomastigote per 20 ml of blood (21, 22). PCR has been very useful in diagnosing acute and chronic Chagas' disease and in monitoring therapy (23).

Cryptosporidiosis

Antibody Testing. In addition to waterborne outbreaks, *Cryptosporidium* infections commonly occur in pediatric and immunocompromised individuals. Serologic tests for antibody for the diagnosis of this infection have not been useful; however, an immunoblot assay has shown specific antigen-antibody banding patterns in individuals presumed to be infected. Using the immunoblot assay as the gold standard, new ELISAs have been shown to be more specific and, with the 27-kDa antigen ELISA, more sensitive than the crude oocyst antigen ELISA currently being used (24). Unfortunately, these reagents are not readily available. Detection of IgG antibodies in sera from patients with *C. parvum* and *C. hominis* has been reported using two sporozoite antigens (15/17 kDa and 27 kDa). Overall responses to the 15/17-kDa antigen complex were stronger and over a greater range than those to the 27-kDa antigen, but declined between 181 and 240 days and were barely detectable thereafter. Responses to the 27-kDa antigen were much weaker but remained detectable for a greater length of time. No differences were detected in either antibody response to infection with *C. hominis* or *C. parvum*. Strong responses with the 15/17-kDa antigen occurred within 6 months of infection (25). A multiplex assay detected IgG antibodies against *Giardia* and *C. parvum* antigens (26).

Antigen Testing. Detection of the organism in stool specimens relies on the use of special staining techniques or fecal immunoassays. Permanent stains normally used in ova and parasite examinations do not adequately stain *Cryptosporidium* spp. A number of commercially available immunoassay kits for antigen detection are available and are more sensitive and specific than routine microscopic examination (27–35) (Table 33.5). Stool specimens may be fresh, frozen, or fixed; however, polyvinyl alcohol-fixed specimens are usually unacceptable for use in the fecal immunoassays. Based on published information, sensitivity and specificity data tend to be comparable for the EIA, FA, and rapid cartridge formats for the detection of *Cryptosporidium* spp. antigen in fecal specimens. In general, PCR has proven to be more sensitive than microscopy and antigen detection immunoassays; however, cost and time remain issues for improvement. Several of the new automated enteric panels include testing for cryptosporidiosis (*Cryptosporidium* spp.); additional information can be found in Appendix 8.

Cyclosporiasis

Currently, there are no manual immunodiagnostic tests available for cyclosporiasis. However, water concentrates have been subjected to community DNA extraction followed by PCR amplification, PCR sequencing, and computer database homology comparison for the detection of *Cyclospora cayetanensis* (37). Also, the BioFire FilmArray Gastrointestinal Panel (bioMérieux, Marcy l'Etoile, France) is designed for the simultaneous detection of 22 different enteric pathogens directly from stool specimens, including *Cyclospora cayetanensis*. This test is a qualitative test and does not provide a quantitative value for the organism(s) in the sample. The FilmArray GI pouch is a closed-system disposable that houses all the chemistry required to isolate, amplify, and detect nucleic acids from multiple gastrointestinal pathogens within a single stool specimen (see Appendix 8).

Giardiasis

Giardia lamblia can cause chronic diarrhea and malabsorption, especially in children. The organism is one of the most easily recognized parasites when detected in the stool; however, variability in the concentration of organisms in the stool makes this infection difficult to diagnose.

Antibody Detection. Serum antibodies to *G. lamblia* have been detected in infected patients; however, because of the lack of sensitivity, they cannot be recommended for diagnosis. After successful therapy, antibodies can be detected for as long as 15 months. Studies show intriguing associations between seroprevalence, outbreak-related laboratory serologic data, and patterns of parasite contamination of drinking water. However, additional studies are required to validate the serologic approach to risk assessment of waterborne parasitic infections at a community level (26, 37).

Antigen Detection. Antigen detection in stool, duodenal fluids, and serum by direct FA, IFA, ELISA, and immunoblotting methods has been reported (27–35). These tests are reliable and as sensitive as routine ova and parasite examinations. Commercial immunoassay kits are readily available (Table 33.5). Users will have to evaluate which kit will be most useful for their own laboratories (38). Some of the methods may require fresh specimens, and stools fixed in preservatives may not be suitable. Also, some of the kits may not detect both trophozoites and cysts of *G. lamblia* but may be selective for only one life cycle stage. In one case, the fecal antigen detection kit for fresh or frozen specimens detects but does not differentiate *Giardia*, *Cryptosporidium*, and *E. histolytica* (35). In a recent assessment of two real-time PCR assays, the Verweij real-time PCR was robust, very sensitive, and suited for use as a first-line diagnostic test for giardiasis (39). Several of the new automated enteric panels include testing for giardiasis [*Giardia lamblia* (*G. duodenalis*, *G. intestinalis*)]; additional information can be found in Appendix 8.

Leishmaniasis

Leishmania spp. are obligate intracellular parasites that are transmitted to the mammalian host by the bites of infected sand flies. Leishmaniasis is mainly a zoonosis, although in certain areas of the world there is primarily human-vector-human transmission. The World Health Organization estimates that 1.5 million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis (VL) occur every year in 82 countries. Estimates indicate that approximately 350 million people are at risk for acquiring leishmaniasis, with 12 million currently infected.

Leishmaniasis refers to a diverse group of diseases, with the spectrum of disease depending on the infecting species. Disease syndromes range from self-healing cutaneous lesions to debilitating mucocutaneous infections to subclinical viscerotropic dissemination to fatal visceral involvement. Since disease presentations vary considerably, a well-defined classification system based on clinical findings is difficult and sometimes confusing. With recent outbreaks in many areas of the world, including Brazil, India, Italy, Spain, Sudan, and Kenya, leishmaniasis has become more widely recognized as an important emerging infectious disease in many developed as well as developing countries.

The taxonomy of *Leishmania* spp. is controversial and reflects the development of multiple parameters on which classification is based. Previously, organisms were classified based on the clinical disease picture and geographic locations; with the development of criteria based on biochemical and genetic data, the taxonomic groupings no longer coincide with recognized disease entities.

Antibody Detection. A number of serologic diagnostic methods have been developed worldwide, although they are not widely available. In general, sensitivities are >90% but specificities are lower. False-positive results can be seen with other infectious diseases, following treatment for VL where antibodies persist for months, and in some asymptomatic patients. There are currently four tests in use, direct agglutination (DA), IFA, CIE, and ELISA. DA appears to be the best choice as a diagnostic tool, is very specific, and does not require expensive equipment or reagents. However, patients may still have positive sera for 1.5 to 5 years after treatment (40). DA has also been adapted to filter paper sampling, and the filter paper eluates compare well with their homologous sera and correlate strongly with antibody titers. However, newer studies using ELISA formats indicate excellent sensitivity and specificity and may be very helpful in early infections.

Currently, IFA is used to detect antibodies to *Leishmania* spp. but cannot differentiate among the various species. However, it can differentiate leishmaniasis from other similar diseases, although some cross-reactivity occurs with *T. cruzi* (the cause of Chagas' disease), leprosy, malaria, and schistosomiasis. Sensitivity is excellent for VL (>90%) but much less so for cutaneous and mucocutaneous leishmaniasis.

The humoral response in patients coinfected with HIV and *Leishmania* is much lower than that in immunocompetent patients; the immunoblot method appears to be sensitive, noninvasive, and specific for the diagnosis of VL in immunocompromised patients (41). IgG titers of 1:16 or greater are considered positive, but provide only suggestive support for the diagnosis of *Leishmania*. The sensitivity of the test is reasonable for VL. The gold standard remains the identification of *Leishmania* amastigotes in stained smears of material aspirated from cutaneous lesions, spleen, bone marrow or other sites. This involves biopsy histology, PCR, and culture.

A previously described ELISA that detects patient antibodies reactive with the recombinant *Leishmania* protein K39 (rK39) has been modified to confirm suspected kala azar (VL) and to detect asymptomatic infection in a community study in Bangladesh. The sensitivity and specificity of the modified ELISA for kala azar were 97.0% and 98.9%, respectively, for sera from the study population (42). Using the rapid rK39 test (Kalazar Detect; InBios International, Seattle, WA) for the detection of VL, results showed a high specificity but only moderate sensitivity; those patients living in an area of endemicity who have a negative test should be evaluated further with additional serological tests and examination of bone marrow aspirates (43).

Antigenic properties of a chimeric recombinant antigen have been evaluated by indirect ELISA using a panel of human and canine sera previously characterized by parasitologic and/or serologic techniques. Chimeric ELISA showed 99% specificity in both human and canine control groups, while the sensitivity was higher in canine VL than in human VL. Results suggest the potential use of this new antigen for routine serodiagnosis of VL in both human and canine hosts (44).

Post-kala azar dermal leishmaniasis (PKDL) is a dermal complication of kala azar. Diagnosis of PKDL is difficult due to the low parasite burden in the lesions. The DA test was compared with those of the rK39 strip test. The sensitivities of the DA test for antileishmanial antibody detection, based on promastigote and amastigote antigens at a cut-off titer of 1:800, were 98.5% and 100%, respectively, with corresponding specificities of 96.5% and 100%. The DA test could correctly detect 100% of polymorphic cases and 95.4% of macular PKDL cases. In comparison, the rK39 strip test was able to correctly diagnose 95.6% of polymorphic and 86.0% of macular PKDL cases. A DA test based on axenic amastigote antigen provided 100% sensitivity and specificity, making it particularly useful for macular PKDL cases, which are often missed by the rK39 strip test. Thus, the DA test is simple, reliable, and inexpensive for PKDL diagnosis and has potential applicability in field conditions (45).

Antigen Detection. Diagnosis of VL is usually confirmed by demonstration of organisms in tissue smears. However, obtaining the specimens may be difficult. Antibody-based diagnostics are limited by their inability to predict active disease. A new LA test (KAtex; Kalon Biological Ltd., Guildford, United Kingdom), which detects parasite antigen in freshly voided and boiled urine, has been evaluated for use with VL patients before and after treatment. KAtex appears to be a promising test, which in this simplified and improved format could be useful in the diagnosis of VL (46, 47). Antigen detection in urine by KAtex is appropriate for primary diagnosis of VL, for monitoring the efficacy of treatment, and for detection of subclinical infection.

Circulating antigens in 35 serum samples from patients with VL were successfully identified using dot-ELISA, giving 100% sensitivity. With the exception of serum samples from patients infected with *Plasmodium vivax*, which has shown cross-reactivity with *Leishmania donovani* antigens, the test appeared to be specific for the detection of circulating leishmanial antigens (48). The dot-ELISA is highly sensitive and specific, inexpensive, and easy to interpret visually, making it an excellent screening test for the surveillance and diagnosis of kala azar in the field (49).

Malaria

Malaria is known to infect more than 250 million individuals throughout the world and kills more than 1 million people a year, most of whom are children. The vector for malaria is the female anopheline mosquito. When the vector takes a blood meal, sporozoites contained in the salivary glands of the mosquito are discharged into the puncture wound. Within an hour, these infective stages are carried via the blood to the liver, where they penetrate parenchymal cells and begin to grow, thus initiating the pre-erythrocytic or primary exoerythrocytic cycle. The sporozoites become round or oval and begin dividing repeatedly. This schizogony results in large numbers of exoerythrocytic merozoites. Once these merozoites leave the liver, they invade the RBCs, initiating the erythrocytic cycle. It has been reported that in *P. vivax* and *P. ovale*, a secondary or dormant schizogony may occur from organisms that remain quiescent in the liver until a later time. These resting stages are termed hypnozoites. Delayed schizogony does not occur in *P. falciparum* and probably does not occur in *P. malariae* or *P. knowlesi*.

Clinical symptoms of malaria include anemia, splenomegaly, and the classic paroxysm, with its cold stage, fever, and sweats. Although the febrile paroxysms strongly suggest infection, many patients who are seen in medical facilities, particularly in the early stages of the infection, do not exhibit the typical fever pattern. They may have fever or several small, random peaks each day. Since the symptoms associated with malaria are so nonspecific, the diagnosis should be considered in any symptomatic patient with a history of travel to an area where malaria is endemic. The typical paroxysm begins with the cold stage and rigors, which last 1 to 2 h. During the next few hours the patient spikes a high fever and feels very hot, and the skin is warm and dry. The last several hours are characterized by marked sweating and a subsequent drop in body temperature to normal or subnormal.

Giemsa-stained thick and thin blood films have been used to diagnose malaria for many years and have always been considered the gold standard. However, a number of alternative methods have been developed, including different approaches to microscopy, flow cytometry, biochemical methods, immunoassay, and molecular methods. The aim of these procedures has been to reduce cost, reduce the need for expensive equipment, increase sensitivity, and provide simple, rapid methods that do not require conventional microscopy.

Antibody Detection. Serodiagnosis is not recommended for the diagnosis of malaria except in cases of transfusion reaction, where the parasitemia is so low that malaria transmission via blood transfusion cannot be documented. For a patient with repeated negative blood smears, serologic testing may be indicated, but the presence of antibody does not correlate with past or present infection.

In regions of low immunity, serologic assays offer an efficient method to identify infectious donors. The recent development of EIAs with improved sensitivity to *P. falciparum* and *P. vivax*, the predominant transfusion threats, has improved the possibility of serologic testing. Although universal serologic screening in regions where malaria is not endemic is not cost-effective, targeted screening of donors identified to be at risk based on travel-based questioning can significantly reduce wastage through reinstatement. Importantly, transfusion safety does not appear to be compromised by this approach, as evidenced by the lack of a documented transmission in France between 1983 and September 2002, where such a strategy has been used since 1976 (50). Malarial parasitemias in healthy donors occur; thus donor malaria-risk strategies must take into account the possibility of such donors. Countries not using malarial antibody screening should consider carefully the collection of donations from donors previously resident in countries of endemicity; temporary deferral is insufficient (51).

IFA should be used only for the diagnosis of idiopathic tropical splenomegaly syndrome (hypertrophic malarial splenomegaly). This test should not be used to diagnose malaria attacks. The gold standard for acute malaria remains the malaria smear (e.g., thin, thick, buffy coat) or antigen capture techniques or PCR. Antibody titers may persist for years after therapy. Titers for tropical splenomegaly syndrome can be extraordinarily high (e.g., 1:125,000) and decline slowly after therapy.

Antigen Detection. The substitution of newer, more expensive drugs mandates rapid, accurate, and inexpensive diagnostic procedures so that directed therapy can be provided. Rapid diagnostic tests for malaria are being used in many areas of the world. Currently, the World Health Organization recommends treatment of confirmed malaria cases only, rather than providing presumptive treatment. Therefore, diagnostic testing becomes an important issue in the management of malaria.

Malaria is a serious parasitic disease, particularly in the nonimmune traveler, and prognosis depends on timely diagnosis. Microscopy remains the gold standard of diagnosis; however, malaria rapid diagnostic tests (RDTs) are increasingly used in nonendemic settings. Malaria RDTs, sometimes called "dipsticks" or malaria rapid diagnostic devices (MRDDs), are used in the diagnosis of malaria by providing evidence of the presence of malaria parasites in human blood. RDTs are an alternative to diagnosis based on clinical grounds or microscopy, particularly where good-quality microscopy services cannot be readily provided; currently, there are more than 200 RDTs available commercially. RDTs are lateral flow immunochromatographic antigen-detection tests which rely on the capture of dye-labeled antibodies to produce a visible band on a strip of nitrocellulose. Extensive lists of malaria RDTs can be found on the following site (http://www2.wpro.who.int/NR/rdonlyres/3B0EEC88-A85A-4C66-BED8-46F13F47D087/0/RDT_selection_criteria2012.pdf; last accessed 20 June 2014). Reported sensitivities vary between different RDT products but are generally good for *P. falciparum*, with RDTs detecting the *P. falciparum* antigen histidine-rich protein-2 (PfHRP2) scoring slightly better than *P. falciparum*-lactate dehydrogenase (PfpLDH)-detecting RDTs. Some detect one or more of the other three species of malaria which infect humans by detecting various other antigens.

RDTs for the fifth human malaria, *Plasmodium knowlesi*, are currently under development. *P. knowlesi* infections can result in a high parasitemia causing severe malaria in many infected individuals. If detected early and treated with appropriate drugs, these patients demonstrate significant clinical improvement. Unfortunately, microscopic methods usually misidentify *P. knowlesi* as the less pathogenic *P. malariae*, leading to inadequate therapy and adverse clinical outcomes. The rapid immunochromatographic card tests have a very low sensitivity in diagnosing knowlesi malaria and can erroneously report *P. knowlesi* as other plasmodia and vice versa (52). With RDTs, patients with *P. knowlesi* could be misdiagnosed as *P. falciparum* with OptiMAL-IT, as *P. vivax* with Paramax-3, and more correctly as non-*P. vivax*/non-*P. falciparum* with BinaxNOW Malaria. There is a need for a sensitive and specific RDT for malaria diagnosis in settings where *P. knowlesi* infections predominate (53).

RDTs are available in three different formats. The simplest form is a dipstick, which is placed in wells containing blood and/or buffer. The nitrocellulose strip may be placed in a cassette, or the third option is to place the strip on a card. Although the cassettes and cards are more expensive, they are considered the easiest to use. As with any diagnostic test system, it is critical that directions be followed and interpretive criteria be thoroughly understood by the user. Also, when diagnosing malaria, patient signs and symptoms should always be considered in addition to the RDT result.

However, these tests do have limitations, including persistence of the PfHRP2 antigen, cross-reactions of *P. falciparum* with the non-falciparum test line and vice versa, and (rare) false-positive reactions due to other infectious agents or immunological factors. False-negative results occur in the case of low parasite densities, which may be a problem with immunologically naive patients who tend to become symptomatic very early with parasitemias as low as <0.002% or lower (100/µl). As an example, the BinaxNOW (Alere, Inc., Waltham, MA) (the only FDA-approved RDT in the United States at the time of this writing) has a maximum sensitivity at 0.1% (5,000/µl). The same test may achieve a high sensitivity in a population in which all infected people have high parasite density (e.g., above 10,000/µl), but achieve low sensitivity in areas where parasite densities are frequently below 200/µl. RDTs are a valuable adjunct to (but not a replacement for) microscopy for the diagnosis of malaria in the returned traveler. In malaria-endemic settings, many of which may be in remote areas, potential problems may include limited temperature control, poor resupply options, and limited training for health workers.

Sensitivity remains a problem, particularly for nonimmune populations. Parasite densities of >100 parasites/µl (0.002% parasitemia) should be detected and are reasonable targets to expect from dipsticks for *P. falciparum* diagnosis. However, this level of sensitivity is at the lower end of the capability of most devices using capture methods for HRP-2 or pLDH. This level of sensitivity is probably as good as clinical laboratory staff in nonspecialized laboratories with limited exposure to malaria cases could expect to provide using microscopy diagnosis. One of the potential benefits would be for inexperienced evening staff personnel, for whom the dipstick identification of a life-threatening parasitemia with *P. falciparum* could prevent a missed infection. However, a negative test could not be accepted and would require confirmation by microscopic examination of both thick and thin blood films for the detection of parasitemia below the present threshold of detection by these rapid tests. Also, there are rare false positives for patients with certain rheumatologic disorders; this is noted in the package insert and has been confirmed by users in this patient population.

Toxoplasmosis

Toxoplasma gondii is capable of infecting a wide range of hosts, including mammals, birds, and reptiles. The first recognized case of human toxoplasmosis was described by Janko, an ophthalmologist, in 1923. The infection can be contracted by ingestion of oocysts from fecal material (animals in the cat family, *Felidae*), ingestion of tissue cysts from raw or inadequately cooked meat, organ transplantation, transfusions, or transplacental transmission. Human infection occurs primarily through the ingestion of raw or undercooked infected meat.

Infection or colonization by *T. gondii* appears to be common in most parts of the world, particularly where cats are found. In the United States, approximately 30% of adults have been exposed to toxoplasmosis; however, the vast majority of these adults did not have recognizable illness.

Congenitally acquired toxoplasmosis can result in serious and debilitating sequelae, including stillbirths, defects seen at birth, and infections in infants who are frequently asymptomatic at birth. In the latter cases, sequelae of congenital infection may arise later in life, most commonly between the first and third decades (54). The incidence of congenital toxoplasmosis is virtually unknown and may be greatly underreported. The clinical severity of congenital toxoplasmosis varies considerably with the trimester of pregnancy when the maternal infection was acquired. Infections contracted during the third trimester result in a large number of congenital infections; however, most infants show no sign of disease. Although infections acquired by the mother in the first trimester result in a smaller number of infected infants, sequelae are the most severe, including a number of stillbirths. Recent evidence suggests that most infants with subclinical infection at birth will develop signs or symptoms of disease (chorioretinitis) as they approach adulthood.

The diagnosis of acute *Toxoplasma* infection can be established by the following: isolation of tachyzoites from blood or body fluids; demonstration of tachyzoites in cytologic preparations or tissue sections; lymph node histologic testing; demonstration of cysts in such tissues as the placenta or in the fetus or neonate; antigen detection; or serologic test results. In older children or adults, the isolation of *T. gondii* from tissues may reflect only the presence of cysts. However, depending on the clinical presentation, the isolation of the organism from lymph node tissue may reflect the presence of tachyzoites.

Antibody Detection. Since isolation of the parasite is not practical for most laboratories, serologic tests are routinely used for diagnosis (Table 33.6). Properly interpreted serologic test results provide excellent supportive evidence for the clinician. Different serologic tests often measure different antibodies that demonstrate distinct patterns of rise and fall with time after infection. A combination of serologic tests is frequently required to establish whether an individual has been infected in the distant past or has been recently infected. An IgM test is used to help determine whether a patient has been infected recently or in the dis-

TABLE 33.6 *Toxoplasma* kits available commercially in the United States[a]

Company[b]	IgG kits	IgM kits
Abbott Laboratories	IMx[c], AxSYM[c]	IMx, AxSYM
Bayer	Immuno 1[c], Advia[c]	Immuno1, Advia
Beckman	ACCESS[c] Avidity	ACCESS
Biokit	LA	
bioMérieux	VIDAS[c]	VIDAS
Bio-Rad	EIA	EIA
Biotecx	EIA	EIA
Biotest	EIA	EIA
Diagnostic Products Corp.	Immulite[c]	Immulite
Diamedix	EIA	EIA
DiaSorin	EIA	EIA
GenBio	EIA, IFA	EIA, IFA
Hemagen Diagnostics	EIA, IFA	EIA, IFA
Meridian Bioscience	EIA, IFA	EIA, IFA
Siemens	EIA	
Inverness Medical Professional Diagnostics (formerly Wampole)	EIA, IFA	EIA, IFA

[a] Information from CDC, FDA. Representative list of available kits.

[b] Abbott Labs, Diagnostics Division, North Chicago, IL 60064; abcam, Bayer Diagnostics, 511 Benedict Ave., Tarrytown, NY 10591; Beckman Coulter, 4300 N. Harbor Blvd., Fullerton, CA 92834; Biokit USA, 113 Hartwell Avenue, Lexington, MA 02173; bioMérieux, 595 Anglum Dr., Hazlewood, MO 63042; Bio-Rad, 4000 Alfred Nobel Dr., Hercules, CA 94547; Biotecx Labs, 6023 S. Loop East, Houston, TX 77033; Biotest Diagnostics Corp., 66 Ford Rd., Suite 131, Denville, NJ 07834; Diagnostic Products Corp., 5700 W. 96th St., Los Angeles, CA 90045; Diamedix Corp., 2140 N. Miami Ave., Miami, FL 33127; DiaSorin, P.O. Box 285, Stillwater, MN 55082; GenBio, 15222 A. Avenue of Science, San Diego, CA 92128; Hemagen Diagnostics, 34–40 Bear Hill Rd., Waltham, MA 02154; Inverness Medical Professional Diagnostics (formerly Wampole), P.O. Box 1001, Cranbury, NJ 08512; Meridian Bioscience, 3471 River Hills Dr., Cincinnati, OH 45244.

[c] ACCESS, Advia, AxSYM, Immulite, IMx, and VIDAS are automated assays.

tant past. Despite the wide distribution of commercial test kits to measure IgM antibodies, these kits often have low specificity and the reported results are frequently misinterpreted. In addition, IgM antibodies can persist for months to more than 1 year. Thus, due to the frequency of misinterpretation of a positive IgM test result, confirmatory testing should be performed.

The Toxoplasma Serology Laboratory of the Palo Alto Medical Foundation Research Institute, Palo Alto, CA (TSL-PAMFRI) offers confirmatory serologic and PCR testing and methods for isolation of the organism. In addition, their medical consultants (Jose G. Montoya, M.D., Jack S. Remington, M.D., and Despina Contopoulos-Ioannidis, M.D.) are available for interpretation of test results and advice on management of patients. This laboratory provides a toxoplasma serological profile (TSP) panel of tests for confirmatory testing. The TSP panel comprises the Sabin-Feldman Dye Test, double sandwich IgM ELISA, IgA ELISA, IgE ELISA, and AC/HS (differential agglutination) test. The TSP has been used successfully to determine whether serological test results are more likely consistent with infection acquired in the recent or more distant past. Extensive test explanations and interpretation can be found in Table 33.7 (55).

Properly interpreted serologic test results provide excellent supportive evidence for the clinician. Classically, the detection of *Toxoplasma* infections has been based on the detection of IgG and IgM antibodies. Diagnostic reagent comparisons indicate that most are comparable in the detection of anti-*Toxoplasma* IgG but vary considerably in the detection of IgM antibodies. It has been shown that specific IgA antibodies are extremely useful in the diagnosis of congenital and acute toxoplasmosis (56). Another test for detecting acute infection involves the antigen binding avidity of IgG antibodies (57).

Individuals should be initially tested for the presence of *Toxoplasma*-specific antibodies to determine their immune system status. A positive IgG titer indicates infection at some point in time. Women who have a positive IgG titer before pregnancy have never been found to transmit *Toxoplasma* infection to the fetus, regardless of the antibody titer. Women who have no demonstrable antibody must be considered at risk for infection. Because the diagnosis of acute toxoplasmosis is usually based on serologic test results and is frequently considered late in the patient's clinical course, serologic titers may have already reached their peak. In these cases, examination of paired sera would be of little benefit and a single high titer may not indicate recent infection, since titers can remain high for years.

TABLE 33.7 *Toxoplasma gondii* serological profile panel (TSP) used in the Toxoplasma Serology Laboratory of the Palo Alto Medical Foundation Research Institute[a]

Test	Explanation	Comments
IgG antibodies, DT	Measured by Sabin-Feldman Dye Test (DT). The DT is a sensitive and specific neutralization test in which live organisms are lysed in the presence of complement and the patient's IgG *T. gondii*-specific antibody. IgG antibodies usually appear within 1 to 2 weeks of the infection, peak within 1 to 2 months, fall at variable rates, and usually persist for life. The titer does not correlate with the severity of illness.	A positive DT indicates the patient has been exposed to *Toxoplasma*. A negative DT essentially rules out prior exposure to *Toxoplasma* (unless the patient is hypogammaglobulinemic). However, in a small number of patients, IgG antibodies might not be detected within 2 to 3 weeks after the initial exposure to the parasite. Also, rare cases of chorioretinitis and toxoplasmic encephalitis in immunocompromised patients have been documented in patients negative for *T. gondii*-specific IgG antibodies.
AC/HS	The differential agglutination test (also known as the "AC/HS test") uses two antigen preparations that express antigenic determinants found early following acute infection (AC antigen) or in the later stages of infection (HS).	Ratios of titers using AC versus HS antigens are interpreted as acute, equivocal, nonacute patterns of reactivity or nonreactive. The acute pattern may persist for 1 or more years following infection. This test has proved useful in helping differentiate acute from chronic infections but is best used in combination with a panel of other tests.
Avidity	The affinity of specific IgG antibodies is initially low after primary antigenic challenge and increases during subsequent weeks and months. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex. The avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated serum.	The avidity test is used as an additional confirmatory diagnostic tool in the TSP for those patients with a positive and/or equivocal IgM test or acute and/or equivocal pattern in the AC/HS test. It is important to remember that avidity testing is a confirmatory test and not the ultimate test for decision-making in pregnant women. Its highest value is observed when laboratory test results reveal high IgG avidity antibodies and the serum is obtained during the time window of exclusion of acute infection for a particular method (i.e., 12 weeks for the Labsystems method, 16 weeks for the VIDAS-bioMérieux method). Low or equivocal IgG avidity antibody results should not be interpreted as diagnostic of recently acquired infection. These low or equivocal avidity antibodies can persist for months to 1 year or longer.
IgM antibodies	Measured by the "double-sandwich" or "immuno-capture" IgM-ELISA method. This method avoids false-positive results due to the presence of rheumatoid factor and antinuclear antibodies.	In most cases, these titers become negative within a few months. However, positive IgM *T. gondii*-specific titers can be observed during the chronic stage of the infection and can persist as long as 12 years after the acute infection. Persistence of these IgM antibodies does not appear to have any clinical relevance, and these patients should be considered chronically infected. IgM results obtained at non-reference laboratories should be confirmed at a *Toxoplasma* reference laboratory; interpretation per confirmatory testing would be interpreted as (i) a recently acquired infection, (ii) an infection acquired in the distant past, or (iii) a false-positive result.
IgA antibodies	IgA antibodies may be detected in sera of acutely infected adults and congenitally infected infants using ELISA or ISAGA methods[b]	IgA antibodies may persist for many months to more than 1 year. For this reason they are not helpful for diagnosis of acute infection in the adult. However, the increased sensitivity of IgA assays over IgM assays for diagnosis of congenital toxoplasmosis represents an advance in diagnosis in the fetus and newborn. In a number of newborns with congenital toxoplasmosis and negative IgM antibodies, the serological diagnosis has been established by the presence of IgA and IgG antibodies.
IgE antibodies	IgE antibodies are detectable by ELISA in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis.	The duration of IgE seropositivity is less than with IgM or IgA antibodies and hence appears useful as an adjunctive method for identifying recently acquired infections.
PCR	PCR is used to detect *T. gondii* DNA in body fluids and tissues. It has been successfully used to diagnose congenital, ocular, cerebral, and disseminated toxoplasmosis. PCR performed on amniotic fluid has revolutionized the diagnosis of fetal *T. gondii* infection by enabling an early diagnosis to be made, thereby avoiding the use of more invasive procedures on the fetus.	PCR has allowed detection of *T. gondii* DNA in brain tissue, CSF, vitreous and aqueous fluid, bronchoalveolar lavage fluid, urine, amniotic fluid, and peripheral blood.

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[a] This testing panel has been clinically relevant in cases of toxoplasmic lymphadenitis, myocarditis, polymyositis, and chorioretinitis and during pregnancy. In cases where sera demonstrate positive IgG and IgM tests, the ability of this panel to differentiate between recently acquired and chronic infections is much better than any single serological test. Information adapted from: *Toxoplasma Serology Laboratory: A Guide for Clinicians* (Palo Alto Medical Foundation; http://www.pamf.org/serology/clinicianguide.html, accessed 20 June 2014). Links to serologic specimens, PCR specimen requirements, and PCR instructions can be found at this site.

[b] ISAGA, immunosorbent agglutination assay.

In acute and congenital toxoplasmosis, IgA and IgM antibodies are the first to be detected. Normally, IgM and IgA antibodies do not cross the placental barrier, and these antibodies have been used as the hallmark of congenital infection. The IgA and IgM titers continue to rise during the first few weeks of infection and may remain positive for a year or more (56). It is possible that the IgA and IgM titers will decrease to undetectable levels by 5 or 6 weeks postinfection; however, this is not always the case, and high levels may indicate infection within the past 3 to 5 months. In some cases, IgM antibodies may be detected by EIA for months to years after acute acquired infection. IgM antibody methods have given both false-positive and false-negative results because of the presence of rheumatoid factor, blocking antibody, and the induction of fetal IgM antibodies against maternal immunoglobulin. The IgM test should be performed using a method such as the IgM capture ELISA to minimize nonspecific reactions (2). It is important to remember that all IgM-positive results should be verified by a reference laboratory such as the CDC or the Toxoplasma Serology Laboratory of the Palo Alto Medical Foundation Research Institute (Palo Alto, CA).

Generally, the demonstration of either IgA or IgM serum antibody is considered diagnostic; however, the use of cord serum is unreliable because of the possible presence of maternal antibody. Both maternal and infant sera should be monitored at the same time. If both sera are positive, the infant's serum should be retested within 1 to 2 weeks. An infant IgA or IgM titer which is stable or continues to rise is diagnostic of infection. Immunocompromised patients and those with ocular toxoplasmosis may have no demonstrable antibody response. AIDS patients with active toxoplasmosis, including central nervous system disease, generally have no IgM antibodies and a low to moderate level of (or occasionally no) IgG antibodies (58). Early treatment of the infection may considerably delay or reduce the normal host immune response.

Antigen Detection. Tests for the detection of circulating antigen have been used for the diagnosis of infection but have not been widely accepted (56, 59). Studies indicate that detection of some low-molecular-weight antigens is diagnostic of reactivated toxoplasmosis (60). These antigens can be detected even with normal dye test titers, and their detection improves the diagnosis of reactivated disease. They may be the result of the release of bradyzoites from ruptured tissue cysts. Because of the difficulties associated with reliable serologic methods for the diagnosis of toxoplasmosis, other methods such as molecular diagnostics have been used for diagnosis. PCR assays are available primarily in specialized research centers (56). PCR is a rapid and specific means of detecting the parasite in infected individuals who have failed to mount an immune response and allows for the infection to be detected much earlier in gestation.

A specific antibody and Western blot analyses have been used to demonstrate the presence of a highly reactive antigen of 36 kDa, not only in the extract of *T. gondii* tachyzoites but also in selected sera of women with confirmed laboratory and clinical signs of recent toxoplasmosis. The 36-kDa antigen was purified from *T. gondii* tachyzoites and human serum by using electroelution from preparative polyacrylamide gels. An ELISA format for the detection of target *Toxoplasma* antigen (TAg-ELISA) in human serum samples has recently been developed. The TAg-ELISA detected the target antigen in 88% of sera of acutely infected women, with 91% specificity among sera from noninfected women. The detection of 36-kDa *Toxoplasma* circulating antigen in human sera appears to be a promising alternative approach for the laboratory diagnosis of active *T. gondii* infection (61).

Trichomoniasis

The diagnosis of *Trichomonas vaginalis*, a sexually transmitted disease, relies primarily on direct microscopic examinations of wet mounts. Trophozoites can be detected in vaginal secretions, urethral specimens, or spun urine. The sensitivity of the direct wet mounts is low (50 to 85%) and can be improved by culture of the organism. DFA and LA reagents are now commercially available, and both can be used on vaginal swab specimens or fluids (62). The automated Affirm VP Microbial Identification Test system is also available and is being used in physician office settings, clinics, and routine clinical laboratories (62) (see Appendix 8). Using oligonucleotide probes, the system can detect *T. vaginalis* and *Gardnerella vaginalis* from a single vaginal swab. Sensitivity has been reported as 100% compared with wet preparation examination but only 80% compared with culture. Although there are still limitations for specimen storage conditions and times, one of the benefits is certainly flexibility for laboratories serving large outpatient populations.

Helminth Infections

Cysticercosis

The diagnosis of cysticercosis usually depends on surgical removal of the parasite and microscopic examination for the presence of suckers and hooks on the scolex. Multiple larvae are frequently present, and the presence of cysticerci in the subcutaneous or muscle tissues indicates that the brain is probably also involved. The calcified larvae can be readily seen on X-ray examination. Computed tomography (CT) scans or magnetic resonance imaging (MRI) techniques may reveal the presence of lesions in the brain. Although neurocysticercosis may be suspected, particularly in a patient living in an area of endemicity, the multiple neurologic syndromes and the various clinical presentations make it difficult to confirm the diagnosis, even with CT or MRI techniques. Human cysticercosis caused by *Taenia solium* is an important public health problem in developing nations and is often the leading cause of epileptic symptoms.

Antibody Detection. Serologic tests used for diagnostic purposes include CF, IHA, IFA, ELISA, immunoblotting, ID, and radioimmunoassay (63). IgG is the predominant immunoglobin detected in patients with cysticercosis; IgA, IgE, and IgM antibodies are of little value in diagnosis and cannot be correlated with the patient's clinical condition. Serodiagnosis of cysticercosis has been a problem because of cross-reactions occurring in patients with coenurus, *Echinococcus*, filaria, and *Taenia saginata* infections.

The CDC enzyme-linked immunoelectrotransfer blot (EITB) using purified *T. solium* antigens has been acknowledged by the World Health Organization and the Pan American Health Organization as the immunodiagnostic test of choice for the diagnosis of cysticercosis (64). It is 100% specific and has an extremely high sensitivity. The test employs purified glycoproteins, making it more sensitive than ELISA, and has eliminated the problems with nonspecificity noted above. Both serum and CSF can be used as diagnostic specimens. The test may be more sensitive with serum than CSF; therefore, there is no need to obtain CSF. For patients with multiple cysts (more than two cysticerci), the test has a sensitivity of 98%. Patients with multiple calcified cysts had a seropositivity of 82%, whereas patients with a single parenchymal cyst had a seropositivity of 50 to 70%. Several private laboratories now offer this test, which was previously available only at CDC.

Several immunoassay kits are also commercially available (Table 33.2). The clinical tests for the diagnosis of cysticercosis cannot be used to distinguish between present and past infections, nor can they be used to monitor the progress of therapy.

The assay of choice for serologic detection of cysticercosis in humans and pigs is EITB, a Western blot assay that relies on the use of chromatography affinity-purified glycoproteins derived from *T. solium* metacestodes. This test facilitates the diagnosis of lesions (usually intracranial) that are compatible with *T. solium* cysts by CT or MRI scans. Although the gold standard test is a tissue biopsy, this procedure is seldom used in the diagnosis of cysticercosis. The sensitivity of the immunoblot depends upon the number and vitality of the cysts. Sensitivity is estimated to be 98% in biopsy-proven cases of neurocysticercosis with two or more cysts. Sensitivity decreases in cases with a single enhancing cyst or calcified cysts (range 28% to 72%). In patients with only subcutaneous cysts, the sensitivity is <50%. The specificity of the test is thought to be 100% and no cross-reactivity is known. Both serum and CSF may be tested, but the highest sensitivity can be achieved in serum.

EITB antigens have now been reproduced in synthetic forms. Four chemically synthesized antigens were assayed individually using ELISA and Western blotting for immunoreactivity against sera from clinically defined neurocysticercosis patients. The sensitivity and specificity of all four antigens using the ELISA format did not meet the standards set by the lentil-lectin-purified glycoproteins (LLGP) EITB, while the results of the Western blot format were comparable to those of the LLGP EITB (65).

Antigen Detection. An ELISA for the detection of antigen secreted by viable *T. solium* metacestodes has been used on pre- and posttreatment CSF samples from Peruvian patients with neurocysticercosis demonstrated by CT and EITB; the sensitivity was 86% (66). There was no difference between antigen levels in CSF taken before or 14 days after therapy. There was a significant positive correlation between antigen levels, the number of live cysts detected by CT, and the EITB results. In contrast, there was a negative correlation with the number of enhancing lesions by CT, supporting the hypothesis that enhancing lesions correspond to a late, dead stage of the parasite. This test approach using antigen specific for viable parasites has great clinical relevance, not only for providing information on the viability of the organisms but also for understanding the pathogenesis of neurocysticercosis before and after therapy.

Antigens and antibodies in sera from humans with epilepsy or taeniasis in an area of Mexico where *T. solium* cysticercosis is endemic have also been evaluated (67). Parasite-specific antigens and antibodies were more common among patients with epilepsy and taeniasis than among the controls. Antigens seemed to be associated with late-onset epilepsy, while the antibodies were associated with subcutaneous nodules. The sensitivities of both tests were low in terms of disease detection; however, the specificity and the positive predictive value of the antigen capture assay were high when used on sera from patients with late-onset epilepsy. Since late-onset epilepsy and neurocysticercosis are associated in areas of endemic infection, antigen capture assays may provide a reliable method of detecting active cases of neurocysticercosis.

Hydatid Disease

In an area of endemic infection, exposure to sheepdogs, the presence of an enlarged liver, the presence of a palpable mass in the right upper quadrant of the abdomen, expectoration of a salty-tasting fluid with hemoptysis, and a round lesion on chest radiographs all support the presumptive diagnosis of hydatid disease in either the liver or lungs. Hydatid cysts in the liver may be confused with congenital liver cysts, amebic abscess, or hepatic tumors. Pulmonary symptoms may be confused with tuberculosis. Hydatid cysts should be considered in patients with abdominal masses for which there is no clearly defined diagnosis. Many asymptomatic cysts are first discovered after radiologic studies.

Antibody Detection. Immunodiagnostic tests have been very helpful in confirming the diagnosis of echinococcosis caused by *Echinococcus granulosus* (cystic disease) or *E. multilocularis* (alveolar disease). Serum antibodies to *Echinococcus* spp. have been detected by IHA, IFA, ELISA, CF, LA, immunoelectrophoresis, immunoblotting, and ID. The immune response depends on the location and viability of the cyst. Individuals harboring calcified, senescent, or dead cysts often have a negative serologic test result. Liver cysts produce a greater serologic response in the patient than do hyaline or lung cysts, and up to 50% of patients with lung cysts are serologically negative. Cysts in the brain and spleen are also associated with a decreased immune response in the host.

A dipstick assay for the serodiagnosis of human hydatidosis using camel hydatid cyst fluid was compared with EITB and Falcon assay screening test-ELISA (FAST-ELISA). For the diagnosis of hydatidosis, the sensitivity, specificity, and diagnostic accuracy of the dipstick assay and EITB were 100, 91.4, and 95.1%, while those of FAST-ELISA were 96.2, 100, and 98.4%, respectively. Since the dipstick assay is easy to perform, has a visually interpreted result within 15 min, and is both sensitive and specific, it could be an acceptable alternative for use in clinical laboratories lacking specialized equipment and the technological expertise needed for EITB and FAST-ELISA (68).

Serologic cross-reactions have been noted in low titers in patients with other parasitic diseases, liver cirrhosis, and collagen diseases (1). A test involving a selected antigen called arc-5 or the 8-kDa antigen has been developed for the specific diagnosis of *Echinococcus* infections. It is recommended that serum be screened by either ELISA or IHA and all positive reactions be confirmed by immunoblotting (2). False-positive reactions with the arc-5 antigen can occur with cysticercosis; however, use of the 8-kDa antigen in the immunoblot assay eliminates this cross-reactivity. Immunoblotting using the 8-kDa antigen provides 99% discrimination between seropositive preoperative cystic hydatid disease cases and cross-reactive noncestode parasitic infections or malignancies.

Cross-reactivity can occur between species. The use of purified *E. multilocularis* antigens with either ELISA or immunoblotting methods has been helpful in differentiating infections caused by the two species (69, 70). Immunoblot tests have been used to monitor the therapy of patients, whereas IHA and ELISA may continue to give positive results for years. Tests for the detection of circulating antigen have been used for diagnosis of infection, but they are not readily available.

Currently EIA with a confirmatory immunoblot is being used. This test plays an important role in the evaluation of suspected hydatid cysts (e.g., liver, lung). Given that aspiration of such cysts is contraindicated, the combination of positive serology and typical CT or MRI appearance of the cyst (e.g., internal septations, daughter cysts, lily pad) is often sufficient to make a secure diagnosis. The sensitivity of the EIA test is high but its specificity is relatively low, with 10–15% false-positive results due to cross-reactions in a wide variety of helminth infections (e.g., cysticercosis) and noninfectious conditions (e.g., cancer, pregnancy, autoimmune diseases). Furthermore, the high sensitivity refers to long-standing hepatic disease only. *E. granulosus* titers may be false negative in several situations; small cysts (e.g., early after infection), intact cysts (i.e., no leakage of the immunogenic cyst fluid), cysts in extrahepatic locations, and heavily calcified cysts (e.g., nonviable). The results of this test should be interpreted with additional caution when an organism other than “classic” *E. granulosus* is suspected on the basis of clinical or epidemiological information (e.g., Arctic *E. granulosus*).

Patients with alveolar hydatid disease are usually serology positive when heterologous *E. granulosus* or homologous *E. multilocularis* antigens are used; crude antigens tend to give nonspecific reactions in alveolar disease, as seen with cystic disease. Immunoaffinity-purified *E. multilocularis* antigens (Em2) used in an EIA method give positive results in >95% of cases of alveolar disease. Tests using Em2 are also more useful for monitoring postoperative conditions than for monitoring posttherapy effectiveness.

Antigen Detection. Efforts to standardize and evaluate the LA test as a simple test for the detection of circulating hydatid antigen in serum are under way. The latex particles were sensitized with hyperimmune hydatid antiserum raised in rabbits. The LA test showed a sensitivity of 72%, a specificity of 98%, a positive predictive value of 93%, and a negative predictive value of 91%. This is the first report of use of the LA test for the detection of hydatid antigen in serum in the diagnosis of cystic echinococcosis (71).

An ELISA using polyclonal antibodies against 24-kDa urinary hydatid protein was moderately sensitive in detecting hydatid antigen in serum and urine. Hence, polyclonal antibodies to 24-kDa urinary hydatid antigen can be used as an alternative source of antibody to detect hydatid antigen in serum, urine, and cyst fluid (72).

Fascioliasis

In *Fasciola hepatica* infections, metacercarial larvae do not cause significant pathologic damage until they begin to migrate through the liver parenchyma. The amount of damage depends on the worm burden of the host. Hyperplasia of the bile ducts occurs, possibly as a result of toxic substances produced by the larvae. Symptoms associated with this migratory phase have included fever, epigastric and right upper quadrant pain, and urticaria, although no eggs are found in the stool until the worms mature, which takes 8 weeks; some patients experience no symptoms.

Once the worms have established themselves in the bile ducts and matured, they cause considerable damage from mechanical irritation and metabolic by-products as well as obstruction. The degree of pathology depends on the number of flukes penetrating the liver. The infection produces hyperplasia of the biliary epithelium and duct fibrosis with portal or total biliary obstruction. The gallbladder undergoes similar pathologic changes and may even harbor adult worms. Adult worms may reinvade the liver parenchyma, causing abscesses.

Multiple stool examinations may be needed to detect light infections. Eggs may be detected in the stool of individuals who have eaten *F. hepatica*-infected liver, thereby yielding an erroneous laboratory result.

Antibody Detection. Serologic diagnosis using *F. hepatica* excretory-secretory antigens has been successfully used in areas of endemicity to detect patient antibodies. This approach includes both ELISA and EITB testing. Antibodies may be detected within 2 to 4 weeks after infection. Sensitivity for the FAST-ELISA was reported to be 95%, while sensitivity for the immunoblot assay using 12-, 17-, and 63-kDa antigens was 100%. However, there does appear to be some cross-reactivity with patients who have schistosomiasis. Serologic test results can be used to monitor treated patients to ensure successful therapy, after which antibody levels drop to normal within 6 to 12 months. Serologic tests are the tests of choice for the diagnosis of acute and chronic fascioliasis when few eggs can be found in the stool or when large populations are being screened. However, the immunodiagnosis of fascioliasis, in Central European regions of low endemicity, has remained a major challenge, and routine diagnostic laboratories are struggling with the selection of a suitable and reliable test.

An ELISA has been developed based on the detection of serum IgG4 antibodies reactive with *F. hepatica* cathepsin L1 (CL1). In an area of high prevalence of fascioliasis, a highly statistically significant correlation was seen when the recombinant and native proteins were used. The assays showed that 38 (59%) of the individuals tested were seropositive while only 26 were positive for *F. hepatica* eggs in the stool. All patients with egg-negative stools were also serologically negative. Also, there was no cross-reactivity with sera from patients with schistosomiasis, cysticercosis, hydatidosis, or Chagas' disease. This study indicates that recombinant CL1 shows excellent potential for use in the development of a standardized assay for the diagnosis of human fascioliasis (73).

In another study of ELISAs using recombinant saposin-like protein 2 and recombinant cathepsin L-1 from *F. hepatica*, results demonstrated excellent performance of re-cSAP2 in relation to specificity (99%), sensitivity (87%), and cross-reactivity (no cross-reactivity with neurocysticercosis and filariasis sera). Based on these results, use of this test in the routine clinical laboratory for the diagnosis of human fascioliasis is warranted (74).

Immunoblotting studies showed that an 8-kDa protein reacts with sera from humans with fascioliasis but not sera from humans with other trematode infections. This result suggests that the 8-kDa protein of *F. hepatica* is one of the diagnostic antigens in human fascioliasis without cross-reaction and may be very useful in the diagnosis of fascioliasis (75).

Antigen Detection. The detection of coproantigens by means of an ES78 sandwich ELISA has been used; coproantigens were detected in all patients with patent infections. The data indicate that this approach is more sensitive than stool examinations for eggs, particularly in early infections (76). A new lateral flow immunoassay also appears to have potential as a point-of-care diagnostic test for the diagnosis of fascioliasis (77).

Filariasis

Lymphatic Filariasis

A wide variety of serologic tests have been used for diagnostic and epidemiologic purposes in cases of human filariasis. Except for use in patients not native to the area of endemicity, immunodiagnostic tests are of limited value. Current tests lack both sensitivity and specificity, and most people from the region of endemicity have a positive serologic response. This response may be due to exposure to nonhuman filarial antigens from infected mosquitoes, and filarial antigens may cross-react with antibodies to other parasitic diseases.

Antibody Detection. The immune response of the filaria-specific IgG4 is considerably increased in patients with active filarial infections. The detection of IgG4 antibodies also reduces the cross-reactivity of nonfilarial antibodies.

IgG2 production appears to be increased in patients with elephantiasis. Studies also suggest that specific antisheath antibodies are associated with an immunologic resistance mechanism that in the endemic community is expressed with highest prevalence in young individuals before the development of patent microfilaremia. However, antibody tests cannot differentiate past from present infection, nor do they show any correlation between antibodies and worm burden.

In the simple and rapid Brugia Rapid (BR) test, an immunochromatographic dipstick is used to detect IgG4 antibodies that are reactive with a recombinant *Brugia malayi* antigen. When sera from 109 individuals with *Brugia* microfilaremias (12 infected with *B. malayi* and 97 infected with *B. timori*) were investigated using the BR test, all were found to be positive. In contrast, all of the 150 sera from individuals with *Onchocerca volvulus* or *Mansonella* infections were found to be negative in BR tests. Some cross-reactions were observed with sera from individuals infected with *Wuchereria bancrofti* (3 of 12 tests positive) and *Dirofilaria* (1 of 9 tests positive) (78).

A rapid-format, simple, and qualitative flowthrough immunofiltration test has been developed for the identification of total IgG antibodies to recombinant filarial antigen WbSXP-1. This test system employs colloidal gold-protein A reagent as the antibody capture reagent. The sensitivity was 90.8% and 91.4% with brugian and bancroftian microfilaremic patients, respectively. The test showed minimum reactivity with *Loa loa* microfilaria-positive sera and no reactivity with *Onchocerca*-positive sera. The test was nonreactive with samples from individuals suffering from other parasitic diseases including schistosomiasis, soil-transmitted helminth infections, and protozoan infections, confirming the potential of this test as a diagnostic tool for both brugian and bancroftian lymphatic filariasis (79).

Some reference laboratories are now using an EIA for antibody detection. Indications for use include several clinical syndromes suggesting a filarial etiology, and screening asymptomatic individuals whose sera and geographic origins or travel make a filarial infection possible. Settings in which testing could be appropriate include generalized itchiness (onchocerciasis), lymphangitis (*W. bancrofti*, *B. malayi*), eosinophilic lung disease (*W. bancrofti*), or a history of intermittent swellings (*L. loa*). This test is of little use in the diagnosis of chronic lymphadema (elephantiasis), hydrocele, and chylurea, all of which typically complicate "burned out" filarial disease. The gold standard for diagnosis of filarial disease remains visualization of adult worms (e.g. *L. loa* eye worm, visualization of mobile worms using ultrasound), or microfilaria (e.g., skin snip). A positive titer (≥1:128) indicates a filaria infection but gives no information with regard to disease state (e.g., acute, chronic, cured). Virtually all residents of filariasis-endemic areas are antibody positive. Anti-filaria antibodies may persist for years after infection. Therefore a single test result must be interpreted with caution when prior infection is possible.

Antigen Detection. Because of the lack of sensitivity in direct-detection methods and problems with obtaining blood specimens within correct time frames, detection of circulating antigens has been used diagnostically. Antigen detection has also been used to monitor therapy. Many of these methods are not yet standardized and are not substitutes for direct-detection methods. Most are not sensitive enough to detect amicrofilaremic individuals who have clinical filariasis.

PCR assays will be a very useful diagnostic tool because they can discriminate between past and present infection and can be used to monitor therapy. In a preliminary evaluation of the diagnostic capability of PCR with diurnally collected sputum from bancroftian filariasis patients, the data were excellent; this approach appears to have excellent potential (80). New approaches for antigen detection also include (i) the ICT card test for serum specimens, (ii) the TropBio ELISA for serum specimens, and (iii) the TropBio ELISA for filter paper specimens; data from all three methods were very promising for their use as diagnostic tools in bancroftian filariasis (81, 82).

Onchocerciasis

The prepatent period of onchocerciasis in humans is 3 to 15 months. Light infections may cause no symptoms, and worms may be free in the tissue; however, in heavy infections, major disease manifestations include dermatitis, onchocercomas (subcutaneous nodules containing adult worms), lymphadenitis, and blindness. Even though there is an inflammatory reaction that causes the formation of a fibrotic capsule (onchocercoma) around the adult worm, the main pathology appears to be directed against the microfilariae.

Antibody Detection. Recombinant antigens for serologic diagnosis can help overcome the problems associated with poor sensitivity and specificity, particularly in areas where other human filarial infections overlap with those caused by *Onchocerca volvulus*. A cocktail of recombinant *O. volvulus* antigens for serologic diagnosis with the potential to predict the endemicity of onchocerciasis infection has been developed (83). When a sentinel population of 5- to 15-year-old individuals was used to compare communities, the serologic index was sufficiently sensitive to measure the changes in endemicity that would be required for control programs. However, serologic testing cannot distinguish between current and past infection and tends to be more important in monitoring specific groups.

It has also been shown that tests based on IgE detection are more specific than those based on IgG detection. However, current tests still lack sensitivity and specificity, and the results do not correlate with past or present infection or with parasite burden.

Four recombinant *O. volvulus* antigens (Ov-FAR-1, Ov-API-1, Ov-MSA-1, and Ov-CPI-1) were tested by luciferase immunoprecipitation systems (LIPS) on a large cohort of blinded sera comprising both uninfected controls and patients with a proven parasitic infection including *O. volvulus*, *W. bancrofti*, *L. loa*, *S. stercoralis*, and other potentially cross-reactive infections. Antibody responses to the four different *O. volvulus* antigens produced unequivocal differentiation between infected and uninfected control sera, with 100% sensitivity and 100% specificity. The multiantigen LIPS assay can be used as a rapid, high-throughput, and specific tool to diagnose individual *O. volvulus* infections, as a sensitive and potentially point-of-care method for early detection of recrudescent infections in areas under control, and for mapping new areas of transmission of *O. volvulus* infection (84).

Antigen Detection. Studies have been reported using recombinant proteins and a dipstick immunobinding assay (DSIA) for the detection of ocular microfilariae, a transblot immunobinding assay (TADA) for the detection of skin microfilariae, and a dot blot immunobinding assay (DIA-BA) for the detection of urinary microfilariae (85). Sensitivities were 100% for DSIA, 82.5% for TADA used on samples of tears, 97% for TADA used on dermal fluids (skin snip), and 96% for DIA used on urine samples.

A recent report discussed the development and evaluation of a DIA-BA based on the biotin-avidin binding system for the detection of *O. volvulus*-specific antigens in body fluids (86). The biotinylated probes were used to detect *O. volvulus*-specific antigens initially blotted onto a nitrocellulose membrane. The smallest amount of blotted antigens detectable by the new test is 0.5, 1, 1, and 2 ng in urine, dermal fluid, tears, and serum samples, respectively. The test was 100% sensitive when used with urine and only 54.76% when used with serum from skin-snip-positive subjects. The specificity of the test was 100%, while the dermal fluid specificity was 97.5%. Also, there was a positive correlation between the color intensities on the blot and the skin microfilaria loads of the individuals. The DIA-BA test could be very useful for mass diagnosis of prepatent, low-level, and high-level infections due to *O. volvulus* (86).

Paragonimiasis

Humans become infected with *Paragonimus* spp. by ingesting uncooked crabs or crayfish containing metacercariae. The metacercariae excyst in the duodenum and migrate through the intestinal wall into the abdominal cavity. The larvae migrate around or through the diaphragm into the pleural cavity and the lungs. They mature to adults in the vicinity of the bronchioles, where they discharge their eggs into the bronchial secretions. Individuals with symptoms of chronic cough, vague chest pains, and hemoptysis who have resided in an area where infections are endemic and have a history of eating raw crayfish or crabs should be suspected of having paragonimiasis. *Paragonimus* eggs can be detected in sputum and in the stool, and concomitant examinations should be performed to improve the overall detection rate. In many individuals in whom the infection is eventually confirmed, small numbers of eggs are present intermittently in the sputum and feces. For patients with light infections, up to seven sputum examinations have been recommended. Frequently, pulmonary paragonimiasis is misdiagnosed as pulmonary tuberculosis. The Ziehl-Neelsen method for detecting mycobacteria destroys *Paragonimus* eggs.

Antibody Detection. In addition to routine diagnostic methods used to recover the characteristic eggs, immunodiagnostic tests have been used to diagnose pulmonary and extrapulmonary infections and to differentiate tuberculosis from paragonimiasis. CF has been used to diagnose active infections since it was noted that the test became negative soon after the death of the worms. An immunoblot test for the detection of antibodies to *Paragonimus westermani* using adult worm homogenates was found to be highly sensitive and specific; this test has been available at CDC since 1988. Only one false-positive result has been obtained with this method: a patient with *Schistosoma haematobium* infection.

Serology may be useful for the diagnosis of stool- and sputum-negative paragonimiasis, *Paragonimus* cutaneous larval migrans, and cerebral paragonimiasis, although the sensitivity in these conditions is unknown. Also, several species of *Paragonimus* infect humans. The sensitivity of the immunoblot assay for infections caused by these other species is unknown. The sensitivity of this assay is ~96% in proven cases *of P. westermani* in Southeast Asians. The specificity is estimated at 99% with low cross-reaction for *S. haematobium*. Antibodies are often undetectable until 3 weeks postinfection. Although CF titers decline rapidly after treatment, no data for antibody persistence in the immunoblot assay are available.

EIA using adult excretory-secretory antigens was found to be very sensitive for detecting parasite-specific IgG and IgE. Pleural effusion fluid may be more suitable than serum for detection of infections. Dot ELISA has also been used to detect parasite-specific antigen in human sera. Monoclonal antibodies to detect species-specific and stage-specific antigens are highly sensitive and specific for the detection of active infections (87). The serology-based assays are available in areas of endemicity or in specialized diagnostic centers. Most of these assays use nonstandardized reagents and have not been used in clinical trials.

An intradermal test is also available for epidemiologic surveys in areas of endemicity; however, positive reactions persist for years after parasitologic cures have been documented. Purification of antigen for intradermal tests does not improve the efficacy of screening for paragonimiasis, probably due to the nature of skin sensitization evoked by many elements of the parasite but not by a few selected components. The crude worm antigen may be valuable for screening a large population, yet the diagnosis should not depend totally on this test. The final diagnosis should be made by repeated sputum examinations over time, a comprehensive patient history, serologic testing, and radiologic testing if necessary.

Paragonimiasis is rarely described in the United States, which has only one indigenous lung fluke, *Paragonimus kellicotti*. This lung fluke is found in a broad range of mammalian hosts, mainly in the midwestern and southern United States within the Mississippi River Basin. Most cases of autochthonous paragonimiasis in the United States caused by native *P. kellicotti* flukes are transmitted by the ingestion of raw or undercooked crawfish, the preferred crustacean intermediate hosts for *P. kellicotti*. However, as immigration from eastern Asia to the United States and travel between Asia and the United States have increased over the years, more imported cases of *P. westermani* infections have been reported in Asian immigrants and in returning travelers. The *P. kellicotti* antigen Western blot test appears to be more sensitive and specific than the CDC *P. westermani* antigen Western blot test for *P. kellicotti* infections in the United States and may also prove useful in monitoring the effectiveness of therapy with praziquantel. New and more sensitive molecular tests are currently under development and may provide improved diagnosis compared with conventional serological technologies.

Schistosomiasis

Humans become infected with *Schistosoma* spp. by penetration of cercariae through intact skin rather than by the ingestion of metacercariae. Once the cercariae have successfully entered the host, the organism is termed a schistosomulum. After about 48 h, the schistosomulum migrates through the tissues and finally invades a blood vessel. On entry into the blood vessels, the schistosomulum is carried to the lungs and then the liver. Once within the liver sinusoids, the worms begin to mature into adults. Depending on the species, the adult worms are found in various veins. The worms form pairs (male and female), with the female lying in the gynecophoral canal of the male. Development of sexual maturity of female schistosomes depends on the presence of mature male worms.

The eggs are immature when first laid and take approximately 8 to 10 days to develop a mature miracidium. Egg deposition takes place intravascularly. Many of the eggs laid are swept downstream, where they become lodged in the microvasculature of the liver and other organs. About half of the eggs swept away by the bloodstream become embedded in the mesenteric venule wall. The presence of eggs in the tissues stimulates granuloma formation; the eggs die, calcify, and are eventually absorbed by the host.

Disease syndromes associated with schistosomiasis are related to the stage of infection, previous host exposure, worm burden, and host response. Syndromes include cercarial dermatitis, acute schistosomiasis (Katayama syndrome), and related tissue changes resulting from egg deposition.

Specific diagnosis of schistosomiasis by detection of eggs in stool or urine specimens is possible only after egg production has begun. Eggs may be found in feces as early as 5 weeks after infection. The ease of egg detection depends on the worm burden and the duration of the infection. Patients with a low worm burden or old (chronic) infections may have very few eggs in their feces or urine, and the infections may not be confirmed due to the use of insensitive diagnostic methods.

Antibody Detection. A large number of serologic tests have been used in the diagnosis of schistosomiasis; however, most have not been particularly useful because of cross-reactions with other helminth infections, continuation of elevated titers long after the completion of successful treatment, and the slow immunologic response of the host. Because of the complex life cycle, a large number of antigens have been used in serologic tests. Only a few have been useful. The most frequently used tests are the circumoval precipitin test, the cercaria-Hullen reaction, IFA, IHA, immunoblotting, EIA, and FAST-ELISA.

Currently at CDC, specimens are first tested by FAST-ELISA with *S. mansoni* adult microsomal antigen. A positive reaction (>8 U/µl of serum) indicates infection with *Schistosoma* species. The sensitivity is 99% for *S. mansoni* infection, 95% for *S. haematobium* infection, and ≥50% for *S. japonicum* infection. The specificity is 99%. Because of the lower sensitivity for *S. haematobium* and *S. japonicum*, immunoblot analyses relevant to the patient's travel history are performed. As with many other parasitic infections, the presence of antibody indicates infection at some time in the past and cannot be correlated with clinical status, parasite burden, egg production, or prognosis.

EIA methods with soluble adult worm or egg antigens for the detection of antibodies have been used as screening tests, with confirmation of infection performed by EITB tests. Individuals infected with schistosomes have an elevated IgE and IgG4 response, as is commonly seen with other helminthic infections. Few studies have investigated the persistence of antibodies over time after therapy. It is possible that high titers posttherapy reflect treatment failure or reinfection. EIA can be used to screen individuals who have travelled to or resided in areas of endemicity and have had freshwater contact and/or have unexplained eosinophilia. In general, the EIA can be used to investigate eosinophilia, abnormal liver function tests, dysentery, hematuria or fever, and hepatosplenomegaly. Positive reactions detected in a patient with definitive filaria, *T. solium*, *Strongyloides*, or *Echinococcus* infections may represent cross-reactions. Anti-*Schistosoma* antibodies may persist for many months after infection; a single test result must be interpreted with caution when prior infection is possible. Seropositivity may be negative early in Katayama syndrome. The performance of this test in individuals infected with related *S. japonicum* and *S. mekongi* is not currently known.

The dipstick dye immunoassay test (DDIA), developed in China for the detection of antibodies against *S. japonicum*, relies on soluble egg antigen (SEA) labeled with a colloidal dye. This assay is not only rapid, simple, and inexpensive but also particularly useful for screening in the field. Sensitivity and specificity results support the notion that the DDIA with *S. japonicum* SEA can safely be implemented for the diagnosis of *S. mekongi*; however, careful interpretation of results is mandatory when samples are obtained from areas where *Opisthorchis viverrini* is also endemic (88).

Antigen Detection. ELISA methods have also been used to detect circulating schistosome antigens in the sera and urine of infected patients and may be the preferred method of diagnosis. In addition to diagnosis of infection, antigen levels have been used to assess disease severity in terms of intensity of infection and to monitor therapy. Individuals treated with praziquantel experienced a rapid clearance of circulating anodic antigen (CAA) and circulating cathodic antigen (CCA); however, in individuals treated with other therapeutic drugs, clearance may take much longer.

A dipstick assay based on the anti-CCA sandwich ELISA, on a nitrocellulose format, can detect CCA in the urine of patients with *S. mansoni* infection with a sensitivity of 92%. The results were at least as good as those obtained using ELISA or the Kato-Katz fecal smear examination. This rapid, simple approach may lend itself to more extensive use in future screening programs (89). The combination of urine CCA and serum CAA for the detection of circulating antigens and the combination of the *S. haematobium* adult worm microsomal antigen (HAMA) FAST-ELISA and the HAMA EITB for detecting antibodies has significantly improved the sensitivity of antibody and antigen detection (90). However, while CCA dipsticks are a good alternative, or complement, to stool microscopy for field diagnosis of intestinal schistosomiasis, they have no proven value for field diagnosis of urinary schistosomiasis (91).

Another reagent strip assay for the diagnosis of schistosomiasis based on detection of parasite antigens in the urine of infected individuals has been evaluated. The test uses the principle of lateral flow through a nitrocellulose strip of the sample mixed with a colloidal carbon conjugate of a monoclonal antibody specific for *Schistosoma* CCA. The strips and the conjugate in the dry format are stable for at least 3 months at ambient temperature in sealed packages, making the test suitable for transport and use in areas where schistosomiasis is endemic (92).

Using an SEA monoclonal antibody, circulating schistosome antigen was undetectable in serum from patients with other parasitic infections and in 94% of serum samples and 84% of urine samples from *S. mansoni*-infected patients. A significant reduction in circulating schistosome antigen levels was detected in serum and urine samples approximately 12 weeks after praziquantel therapy (93). In another study using a 63-kDa circulating antigen, the antigen was detected in 92% of urine samples from 330 *S. mansoni*-infected individuals, with a 16% false-positive result among 130 noninfected individuals (94).

In reviewing the current status of schistosomiasis diagnosis, the data obtained show that promising new diagnostic tools, especially for *Schistosoma* antigen and DNA detection, which are characterized by high sensitivity and specificity, are being developed. In combination with international funding initiatives, these tools may result in significant steps forward in successful surveillance and disease elimination. Hopefully, more efficient tests will become accessible and self-sustainable for control programs in endemic countries.

Strongyloidiasis

Human infection with *Strongyloides stercoralis* is acquired by skin penetration by filariform larvae (infective larvae) from the soil. These larvae are long and slender and may remain viable in soil or water for several days. After penetration of the skin, they are carried via the cutaneous blood vessels to the lungs, where they break out of the pulmonary capillaries into the alveoli. They then migrate via the respiratory tree to the trachea and pharynx, are swallowed, and enter the mucosa in the duodenum and upper jejunum. Development usually takes about 2 weeks, after which the females begin egg production. The eggs usually hatch, and the rhabditiform larvae (noninfective larvae) pass out of the intestinal tract in the feces. These larvae are passed out onto the soil in the feces and develop into free-living male and female worms, eventually producing infective filariform larvae (eggs, noninfective larvae, and infective larvae). In temperate climates, the free-living male and female worms do not develop; however, the rhabditiform larvae that pass out in the stool develop into the filariform (infective) larvae, which are then ready to infect the next host through skin penetration.

In situations in which autoinfection occurs, some of the rhabditiform larvae within the intestine develop into filariform larvae while passing through the bowel. These larvae can then reinfect the host by (i) invading the intestinal mucosa, traveling via the portal system to the lungs, and returning to the intestine or (ii) being passed out in the feces and penetrating the host on reaching the perianal or perineal skin.

Pathology present in strongyloidiasis can vary, both in severity and in the areas of the body involved. Some individuals remain totally asymptomatic, with the only abnormal clinical finding being a peripheral eosinophilia. Considering the life cycle of the parasite, the three areas of primary involvement would be the skin (cutaneous), the lungs (pulmonary), and the intestinal mucosa (intestinal).

Confirmation of the infection depends on recovery and identification of the adult worms, larvae, or eggs from the stool, duodenal material, or sputum. Fecal specimens may not be positive even after the routine ova and parasite examination, including the concentration procedure, is performed. The numbers of larvae found in the stool also vary from day to day.

Antibody Detection. Although not routinely available, EIA and immunoblot methods have been investigated for use in both the diagnosis and postchemotherapy evaluation of human strongyloidiasis. These methods appeared to be both sensitive and specific, and within about 6 months after therapy they demonstrated a significant decrease in antibody levels. However, in cases where treatment was not effective, the individuals did not show a significant fall in antibody titers. More detailed studies to determine appropriate intervals for serologic evaluation and criteria for successful cure are required before these tests become more widely used.

Currently, EIA, with a sensitivity of 84 to 92%, is recommended. Some patients may be negative, particularly those who are carriers (8 to 16%), and antibody test results cannot be used to differentiate between past and current infection. It is interesting that immunocompromised patients still tend to have detectable IgG antibodies, in spite of their immune system deficiencies.

Strongyloides ratti larval extract was used for the standardization of ELISA to detect genus-specific IgE in humans with strongyloidiasis. A significant positive correlation was found between levels of *Strongyloides*-specific IgE and total IgE in sera from patients with strongyloidiasis. *S. ratti* heterologous extract could be a useful tool for detecting genus-specific IgE by ELISA, thus helping characterize the immune response profile in human strongyloidiasis (95).

A recombinant antigen developed by the National Institutes of Health has been used in an EIA for antibody detection. These tests can be used to screen persons with unexplained eosinophilia, epigastric symptoms, or larva migrans on the trunk. Screening is also appropriate for individuals who have lived or traveled in the tropics or those who will undergo immunosuppressive therapy (transplants, chemotherapy, high-dose corticosteroids). In certain circumstances, positive samples can be tested by immunoblot. This confirmatory assay shows good specificity (98%) with a sensitivity equivalent to the EIA (~95%).

In one study, three immunoassays were evaluated—including the recently released InBios Strongyloides Detect IgG ELISA (InBios International, Inc., Seattle, WA), the SciMedx Strongyloides serology microwell ELISA (SciMedx Corporation, Denville, NJ), and the luciferase immunoprecipitation system (LIPS) assay performed at the National Institutes of Health—for their detection of IgG antibodies to *S. stercoralis*. The overall percent agreement of the InBios, SciMedx, and LIPS assays was comparable at 87.1%, 84.2%, and 89.1%, respectively. However, while the two commercially available ELISAs perform equivalently, neither should be used independently of clinical evaluation to diagnose strongyloidiasis (96).

Antigen Detection. Polyclonal rabbit antiserum was raised against *S. ratti* excretory/secretory (E/S) antigen and utilized to develop an antigen capture ELISA. The assay enabled detection of subpatent rodent *S. ratti* and human *S. stercoralis* infection. No cross-reactivity was observed with purified E/S from *S. japonicum* or the hookworms (*Ancylostoma caninum*, *A. ceylanicum*), nor with fecal samples collected from rodents harboring *Trichuris muris* or *S. mansoni* infection. *Strongyloides* coproantigens that appear stable when frozen as formalin-extracted fecal supernatants stored at –20°C remained positive up to 270 days of storage, whereas supernatants stored at 4°C tested negative. These results indicate that diagnosis of human strongyloidiasis by detection of coproantigen has great potential in the diagnosis of strongyloidiasis (97).

Toxocariasis

Toxocara spp. are non-human-derived ascarids that are capable of undergoing limited development in the human host. Infections with the organism have been classified into two categories: visceral larva migrans (VLM), which is a systemic infection occurring in patients with a mean age of 4.6 ± 3.6 years; and ocular larva migrans (OLM), which also occurs in children. However, concurrent infection with VLM and OLM in the same patient is rare. Eosinophilia, which is pronounced in VLM, is almost absent in OLM. The average age of patients with OLM is also older than that of patients with VLM. The term neural larva migrans is often used when the central nervous system is involved.

Antibody Detection. The clinical diagnosis of toxocariasis can be very difficult, and only fairly recently has a reliable immunodiagnostic test been developed. Serologic test results must be interpreted with care because of the prevalence of asymptomatic infection and the age and socioeconomic status of the individual (98). Previous serologic tests had involved adult- or larval-stage somatic extracts and whole or sectioned larvae. The EIA uses third-stage-larva E/S antigen and is superior to all other serodiagnostic tests. The test is highly specific and does not give cross-reactions with sera obtained from patients infected with other commonly occurring human parasites.

Even though EIA is an extremely sensitive test, the diagnostic predictive value for VLM is significantly different from that for OLM (2) (91). A titer of 1:32 is considered diagnostic for VLM (sensitivity, 78%), whereas a titer of 1:8 is considered diagnostic for OLM (sensitivity, 90%). The specificity is >90% at a titer of ≥1:32. This lower predictive titer for OLM raises the possibility of a false-positive diagnosis in a patient who has an asymptomatic *Toxocara* infection and ocular disease due to other etiology. In addition to serum antibody, vitreous antibody can be measured. Titers in the vitreous fluid were equal to or greater than the serum titers. Measurement of ocular fluid antibody should increase the specificity of the EIA, yielding a better definitive diagnosis for the patient. This test remains the definitive test for the diagnosis of *Toxocara* VLM, despite the lack of good studies of its sensitivity and specificity. The test can be used in the investigation of asymptomatic eosinophilia. The role of this assay in defining the etiology of suspected retinal toxocariasis is not clear.

There is no gold standard test with which the performance of serologic assays for *Toxocara* can be compared; larvae are very rarely identified in the human host. Furthermore, the titers obtained in individuals with clinical VLM vary widely. The sensitivity and specificity estimates (86% and 98%, respectively) apply to infections with *T. canis*. The performance of this test in individuals infected with related species (e.g., *T. leonis*) is not currently known. Cross reactions can occur with other nematode infections (trichinosis, filariasis, strongyloidiasis etc.). The IgG response in toxocariasis is likely to be proportional to the number of infecting larvae.

Trichinosis

Human infection with *Trichinella spiralis* is initiated from the ingestion of raw or poorly cooked pork, bear, walrus, or horse meat or meat from other mammals (carnivores and omnivores) containing viable, infective larvae. The tissue is digested in the stomach and the excysted larvae invade the intestinal mucosa, develop through four larval stages, mature, and mate by the second day. By day 6 of infection, the female worms begin to deposit motile larvae, which are carried by the intestinal lymphatic system or mesenteric venules to the body tissues, primarily striated muscle. Deposition of larvae continues for approximately 4 weeks, with each female producing up to 1,500 larvae in the nonimmune host.

The very active muscles, which have the greatest blood supply—including the diaphragm, muscles of the larynx, tongue, jaws, neck, and ribs, the biceps, and the gastrocnemius—are invaded. The cyst wall results from the host immune response to the presence of the larvae, and the encysted larvae may remain viable for many years, although calcification can occur within less than a year. As few as 5 larvae per g of body muscle can cause death, although 1,000 larvae per g have been recovered from individuals who died from causes other than trichinosis.

Symptoms that may develop within the first 24 h include diarrhea, nausea, abdominal cramps, and general malaise, all of which may suggest food poisoning, particularly if several people are involved. Studies also indicate that the diarrhea can be prolonged, lasting up to 14 weeks (average, 5.8 weeks), with few or no muscle symptoms.

During muscle invasion, there may be fever, facial (particularly periorbital) edema, and muscle pain, swelling, and weakness. The extraocular muscles are usually the first to be involved, followed by the muscles of the jaw and neck, limb flexors, and back. Muscle damage may cause problems in chewing, swallowing, breathing, etc., depending on which muscles are involved. The most severe symptom is myocarditis, which usually develops after the third week; death may occur between the fourth and eighth weeks. Other severe symptoms, which can occur at the same time, may involve the central nervous system. Although *Trichinella* encephalitis is rare, it is life-threatening.

The diagnosis of trichinosis is based on a combination of epidemiology and clinical findings (muscle symptoms, diarrhea, eosinophilia, raised creatine kinase and a high positive or rising *Trichinella* antibody titer). A positive muscle biopsy for *Trichinella* larvae is the gold standard test but is rarely required because the sensitivity of the clinical and laboratory findings listed above is excellent.

Antibody Detection. Serologic tests are very helpful, with the standard two being EIA and bentonite flocculation, which are recommended for trichinosis. EIA detects antigen first and is used for routine screening, and all EIA-positive specimens are tested by bentonite flocculation for confirmation. A positive reaction in both tests indicates infection with *T. spiralis* within the previous few years. Often, antibody levels are not detectable within the first month postinfection. The titers tend to peak in the second or third months postinfection and then decline over a few years. Positive reactions are detected at some point during disease progression in 80 to 100% of all patients with symptomatic disease; detectable antibody usually appears between 3 and 5 weeks after infection.

The EIA sensitivity and specificity estimates (93% and 85%, respectively) apply to infections with *T. spiralis*. The performance of this test with other species (e.g., *Trichinella nativa*) appears to be similar. Cross-reactivity can occur with other helminth infections (e.g., filaria, *Strongyloides*). The IgG response to *T. spiralis* and *T. nativa* infection tends to be slow and is proportional to the number of larvae ingested. Anti-*Trichinella* antibodies can be detected at 2 weeks in most individuals who have consumed a large number of cysts. Less heavily infected individuals may take up to 3 to 4 weeks to mount a detectable IgG response. Antibody titers peak 3 to 6 months after infection and then fall progressively even without treatment. Since low titers may persist for many years after infection, a single test result must be interpreted with caution when prior infection is possible.

Antigen Detection. A modified double-sandwich ELISA has been developed using polyclonal antibodies raised in rabbits and guinea pigs against larval somatic antigens of *T. spiralis*. The first detection of coproantigen was as early as the first day postinfection; the level of coproantigen gradually increased to reach its peak on the seventh day and then decreased to disappear completely during the third week postinfection. Another test, the coagglutination test, was used, and this test confirmed the previous results. Results suggest that the coproantigen detection test could be used to confirm ongoing early *T. spiralis* infection (99).

Intradermal Tests

In the absence of reliable serologic diagnostic tests, skin tests have been used to provide indirect evidence of infection. Most skin tests have been used primarily for research and epidemiologic purposes. Two of the more widely used skin tests are the Casoni and Montenegro tests. In many cases, the antigens used are difficult to obtain and are not commercially available. The antigens are usually crude extracts that have not been standardized and are neither highly sensitive nor specific. They may provoke an immune response that complicates further serologic testing, and there is always the danger of provoking an anaphylactic reaction. In addition, there are ethical questions related to giving patients injections of nonstandardized foreign protein, particularly if the antigens were derived from *in vivo* materials.

Casoni Test

In the past, the Casoni test was the only means of diagnosing exposure to hydatid disease. This intradermal test, in which hydatid cyst fluid is used as the antigen, has many advantages as a result of its simplicity; its nonspecificity has been its major limitation. Diagnostic sensitivity was limited in patients who had intact or hyaline cysts. An immune response was detected more frequently in patients with liver cysts than in those with lung cysts.

False-positive reactions have occurred in patients with other parasitic and nonparasitic diseases. The Casoni antigen may also sensitize the patient, leading to antibody production, and anaphylactic reactions have also been reported. This test is rarely used for diagnostic purposes because of the ethical concerns addressed above.

Casoni's skin test and IHA are still used in Turkey. For 120 patients retrospectively studied during 1997 to 2004, results indicated that an immediate skin reaction to crude hydatid antigens was more useful than IHA (100).

Montenegro Test

The Montenegro test uses formalinized promastigotes of any species of *Leishmania* to evoke a delayed hypersensitivity reaction in infected patients. In some areas, it is the method of choice for diagnosis because of its simplicity.

Patients infected with *Leishmania donovani* (kala azar) have a negative Montenegro test during active disease. The test becomes positive after successful treatment or when there is a spontaneous cure. Problems similar to those mentioned above for the Casoni test have occurred with the Montenegro test. The skin test may induce antibody production and may also cause anaphylactic reactions.

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34

Histologic Identification of Parasites

Protozoa
Amebae
Flagellates
Ciliates
Coccidia
Microsporidia
Helminths
Nematodes
Cestodes
Trematodes
Blood Parasites
Malaria
Leishmania
Trypanosomes
Filarial Nematodes
Appendixes

Often, histology preparations are submitted to the clinical laboratory for review, consultation, or confirmation. Sections of tissue that contain parasites may be seen infrequently in many pathology departments; thus, consultation services are needed (1–6).

This chapter is designed not as a comprehensive presentation of histologic parasitology but rather as a review of parasite morphology, potential sites in the human host, any recommended special processing or stains, and additional helpful hints. The information is arranged in table form according to body site (see Appendix 34.1).

Specific organisms are listed within parasite groups (protozoa, helminths, etc.). Appendix 34.1 does not include organisms that have been reported as only rarely infecting humans. Some of these more rare infections are represented by so few cases that a physician may never see one (see chapter 29). The photographs in Fig. 34.1 to 34.73 illustrate representative parasitic infections in various body tissues. Although most of the specimens are from human tissues, a few are from animals to illustrate a particular parasite and representative body tissue.

Although a great deal of information is contained in this chapter, there has been no attempt to illustrate every organism and every possible tissue involved. Also, identification of parasite adults or immature forms depends on specimen submission and subsequent preparation. If the organisms and tissues are not well preserved, identification may be difficult or impossible. Distinguishing actual organisms from artifact material may also complicate the process.

Most diagnostic procedures in medical parasitology do not require extensive patient information; however, the examination of tissues and final identification of any organisms present can be simplified by reviewing as much information as possible about the patient. Specifics would include current symptoms, recent health status, health status of family members, place of residence, food preferences, travel history, outdoor activities, animal exposure, and any possible unusual environmental exposures. If specific lesions have been identified, the source of the tissue(s) must be identified, as well as the method by which the specimen was obtained. It is particularly relevant to know anything about the status of the specimen regarding adequate fixation and/or any lag time prior to fixation that might influence the overall morphology.

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Some general descriptions are provided with diagrams to illustrate specific morphologic criteria used for parasite identification. Because the protozoa are unicellular organisms with one or more nuclei and various organelles, their simplicity does not require extensive descriptive material. However, the identification of various helminths and arthropods requires a more thorough understanding of parasite morphology and appropriate terminology (Appendix 34.2). Appendixes 34.3 to 34.5 contain information on staining characteristics, nuclear and cytoplasmic stains, and specific stains for carbohydrates. This information provides some basic guidelines for the understanding of how various stains are categorized, used, and interpreted. Appendixes 34.6 and 34.7 indicate the locations within the body where human protozoal and helminth parasites are found. Table 34.1 contains very specific information on the musculature of nematodes and can be found following Fig. 34.20 through 34.22. One can also refer to the excellent references provided with this chapter.

Protozoa

Amebae

Entamoeba histolytica

Infection of *E. histolytica* is initiated following ingestion of the cysts of *E. histolytica*; excystation of the cysts in the intestinal lumen produces trophozoites, which colonize the large intestine. The trophozoite may remain confined to the intestinal lumen, feeding on bacteria and cellular debris. Galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)-specific lectins are used by the trophozoites to aid adherence to colonic mucins. At some point, cysts are formed and subsequently excreted in stool. Colitis results when the trophozoites penetrate the intestinal epithelium. The mechanism involved in superficial epithelium erosion is complex; once the protective mucus barrier has been broken down, trophozoites of *E. histolytica* will adhere and produce damage and lysis of epithelium cell (Fig. 34.1 to 34.5) (7). The first findings seen at sigmoidoscopy include nonspecific thickening of the mucosa or pinhead-size micronodules. The pathological range of amebic colitis extends from mucosal thickening with discrete ulcer formation, separated by regions of normal colonic mucosa, to diffusely inflamed and edematous mucosa. Typical intestinal amebic ulcers are found in the colon (primarily in the cecum), sigmoid colon, and rectum. Nodular and irregular ulcers have been described. Nodular lesions appear as small (0.1- to 0.5-cm), rounded, slightly elevated areas of the mucosa with irregular necrotic centers surrounded by a rim of edematous tissue. The necrotic center may appear depressed or hemorrhagic but more often is filled with a yellowish mucoid material. Irregular or serpiginous ulcers 1 to 5 cm in length are usually found in the cecum and ascending colon. These ulcers are characteristically shallow with broad, elevated margins and are filled with fibrin. Congestion and edema of the narrow strips of uninvolved mucosa and edematous thickening of the entire intestinal wall are frequently observed accompanying these large ulcers. Both types of intestinal ulcers are often present in the same patient.

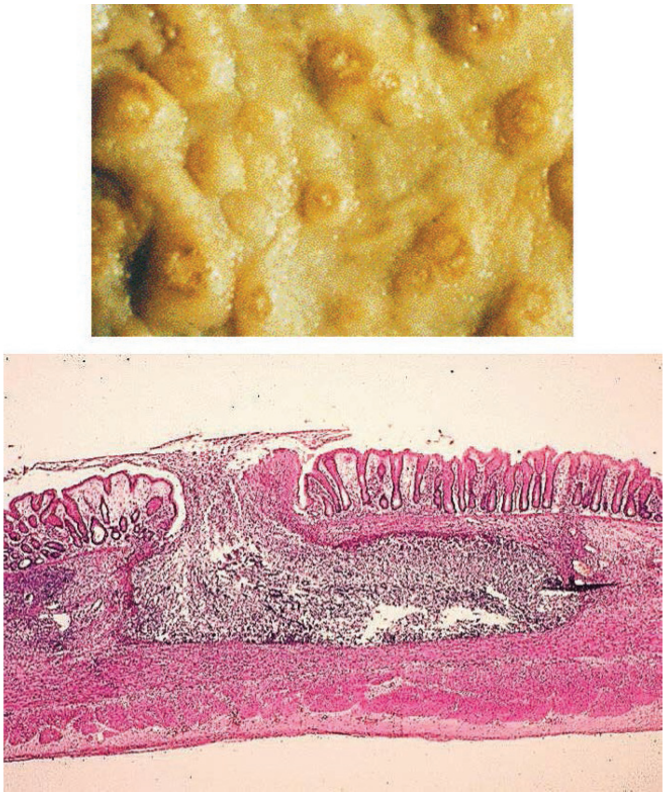

Figure 34.1 Amebic intestinal ulcers. (Upper) Intestinal ulcers; these may coalesce under the surface. (Lower) Note the "flask shape" (small opening on the mucosal surface and a larger area of destruction below the surface); the serosa is edematous and hyperemic. Multiple ulcers may develop and coalesce. Organisms would be found in the healthy tissue border, not in the necrotic material within the ulcer. (Courtesy of the CDC Public Health Image Library, Mae Melvin.)
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Mild to moderate neutrophil infiltration is seen within and around capillaries, but small collections can also be identified beneath the surface epithelium. Lymphoid aggregates show reactive hyperplasia with numerous histiocytic cells. Trophozoites are present in small numbers in the surface exudate. As the lesion progresses, there is a mucopenic depression due to loss of mucin from the surface and

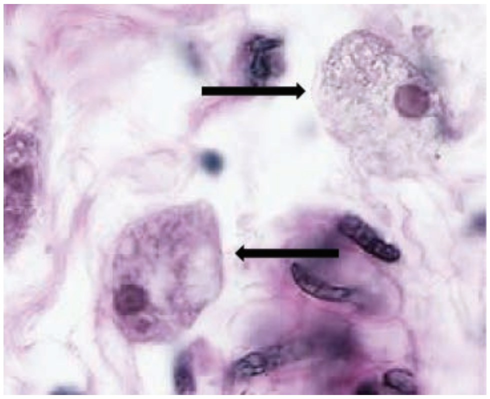

Figure 34.2 Amebic intestinal ulcer. *Entamoeba histolytica* trophozoites in colon tissue (arrows); note the nucleus in each trophozoite (central karyosome); H&E stain (courtesy of the CDC Public Health Image Library).
doi:10.1128/9781555819002.ch34.f2

from glandular epithelial cells, which decrease in height from columnar to cuboidal and flattened dividing cells. A larger neutrophil infiltrate is evident, and plasma cells, eosinophils, macrophages, and lymphocytes can also be identified. Organisms may be present in large numbers on the luminal surface but are particularly abundant adjacent to the sites of epithelial lysis. They are surrounded by a proteinaceous exudate composed mainly of fibrin, mucin, erythrocytes, and occasionally neutrophils and mononuclear cells. Invasion of the colonic and cecal mucosa by *E. histolytica* begins in the interglandular epithelium, forming the early invasive lesions with superficial ulceration (Fig. 34.2, 34.3).

Figure 34.3 Amebic intestinal ulcer. *Entamoeba histolytica* trophozoites in colonic ulcer, demonstrating a nucleus with tiny, centrally placed karyosome (arrow). Brown-Hopps stain (Armed Forces Institute of Pathology photograph).
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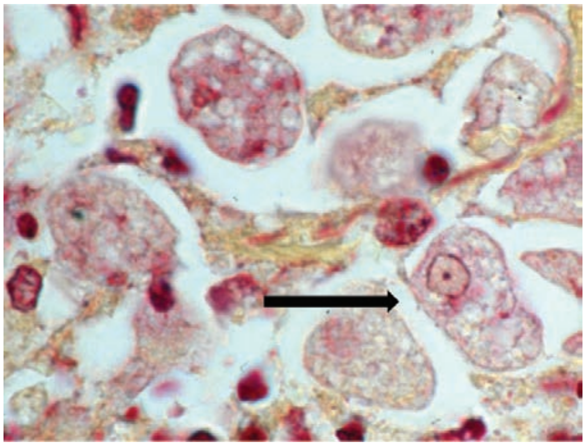

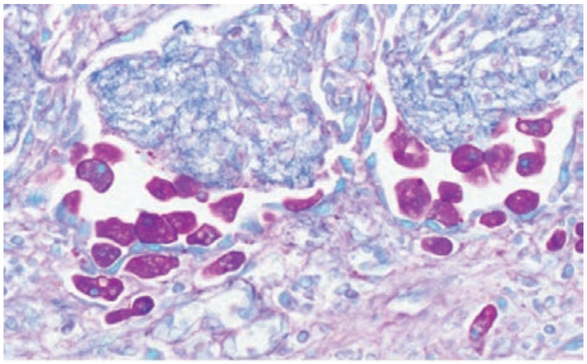

Figure 34.4 Amebic lung section. *Entamoeba histolytica* trophozoites in lung tissue, PAS stain. Note the trophozoites are bright pink and can easily be seen. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch34.f4

The late invasive lesion is characterized by extension of the mucosal ulcer deep into the submucosa. Once the interglandular epithelium has been invaded by trophozo-

Figure 34.5 Amebic liver abscess. (Upper) Gross image of liver abscess caused by *Entamoeba histolytica*. Note the sunken areas of the abscess. (Lower) Gross image of liver abscess, cut specimen. (Courtesy of the CDC Public Health Image Library, Mae Melvin.)
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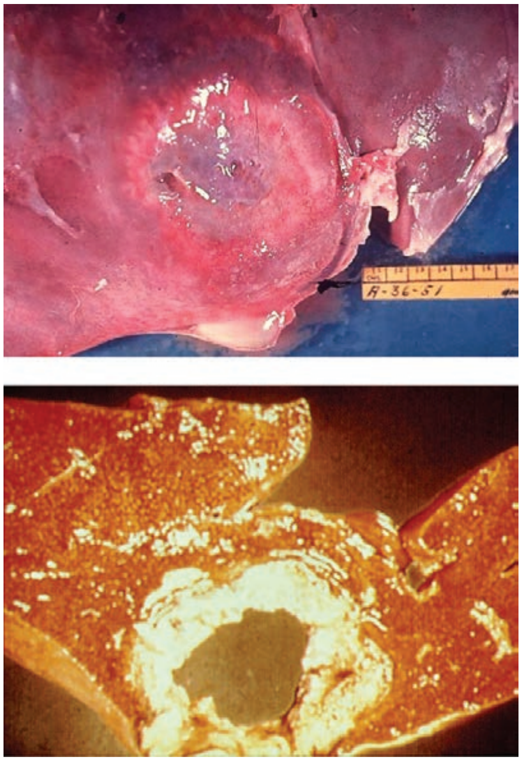


ites, the underlying tissue offers little resistance, allowing extension of the ulcer laterally, creating the classical flask-shaped amebic ulcer. Histopathology shows necrotic areas and vascular congestion with minimal inflammation in contrast with the extension of the lesion. Trophozoites may be found in the surface layer of the ulcers; bacterial infection causing deficiency of blood supply leads to necrosis, hemorrhage, and gangrene and subsequent perforation of the intestinal wall.

On rare occasions, *E. histolytica* trophozoites enter the bloodstream and disseminate to other body sites (Fig. 34.4 and 34.5). Amebic liver abscess is the most frequent manifestation of extraintestinal amebiasis. Amebic liver abscesses are massive fluid-filled cavities that result from extraintestinal spread of the parasite and can be fatal if untreated.

Histological analysis of liver sections from human amebic liver abscess patients consistently shows massive accumulation of inflammatory cells (primarily neutrophils and macrophages) within the abscess. While these immune cells represent the first line of defense against microorganisms, such an overwhelming immune response and the antimicrobial factors released by inflammatory cells could damage the host tissues as well.

Acanthamoeba spp.

The onset of corneal infection with *Acanthamoeba* spp. can vary tremendously; however, two factors often appear to be involved: trauma and contaminated water. When corneal abrasions occur, the disease process is usually more rapid, with ulceration, corneal infiltration, iritis, scleritis, severe pain, hypopyon (pus in the anterior chamber), and loss of vision. Decreased corneal sensation has contributed to the misdiagnosis of *Acanthamoeba* keratitis as herpes simplex keratitis; this mistake can also be attributed to the presence of irregular epithelial lesions, stromal infiltrative keratitis, and edema, which are commonly seen in herpes simplex keratitis.

Although cutaneous infections are more common in patients with AIDS, regardless of the presence or absence of central nervous system (CNS) involvement, they have also been seen in immunocompetent patients. The disease includes the presence of hard erythematous nodules or skin ulcers. The early lesions appear as firm papulonodules that drain purulent material; these lesions then develop into nonhealing indurated ulcers. Histology reveals necrosis surrounded by inflammatory cells, vasculitis, and the presence of trophozoites and cysts of the amebae (Fig. 34.6).

Granulomatous amebic encephalitis is characterized by confusion, dizziness, drowsiness, nausea, vomiting, headache, lethargy, stiff neck, seizures, and sometimes hemiparesis. Within the CNS, the cerebral hemispheres are the most likely tissue to be involved. There may be edema and hemorrhagic necrosis within the temporal, parietal, and occipital lobes. A chronic inflammatory exudate can be seen over the cortex, mainly of polymorphonuclear leukocytes and mononuclear cells. Unlike in primary amebic meningoencephalitis caused by *Naegleria fowleri*, both trophozoites and cysts are found throughout the tissue. Granulomatous inflammation, necrosis, and thrombosed vessels are seen in the brain. Multinucleated giant cells forming granulomas can be seen in immunocompetent patients, while granuloma formation is generally poor or lacking in immunocompromised individuals.

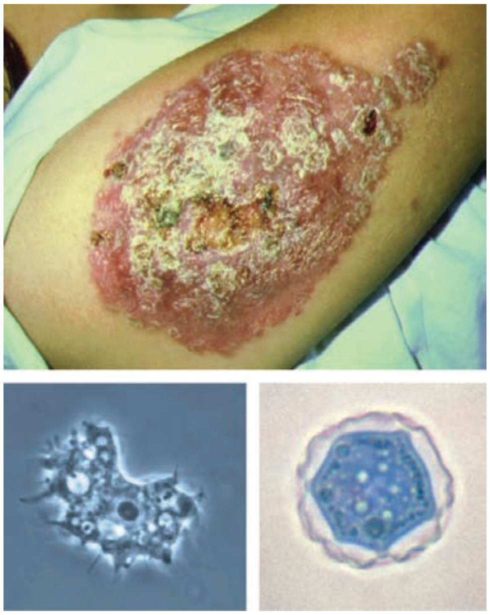


Figure 34.6 *Acanthamoeba* spp. (Upper) Cutaneous abscess on arm; nonhealing ulcer, possibly as a result of a human bite wound, that was not recognized as being caused by *Acanthamoeba* spp. Therapy with routine antibiotics was ineffective. (Courtesy of George Healy, Centers for Disease Control and Prevention.) (Lower) *Acanthamoeba* trophozoite (left), cyst (right).
doi:10.1128/9781555819002.ch34.f6

Naegleria fowleri

Primary amebic meningoencephalitis caused by *N. fowleri* is an acute, suppurative infection of the brain and meninges. With extremely rare exceptions, the disease is rapidly fatal in humans. Extensive tissue damage occurs along the path of amebic invasion; the nasopharyngeal mucosa shows ulceration, and the olfactory nerves are inflamed and necrotic. Hemorrhagic necrosis is concentrated in the region of the olfactory bulbs and the base of the brain, with pockets of amebic trophozoites within edematous and hemorrhagic CNS tissue. Organisms can be found in the meninges, perivascular spaces, and sanguinopurulent exudates (Fig. 34.7).

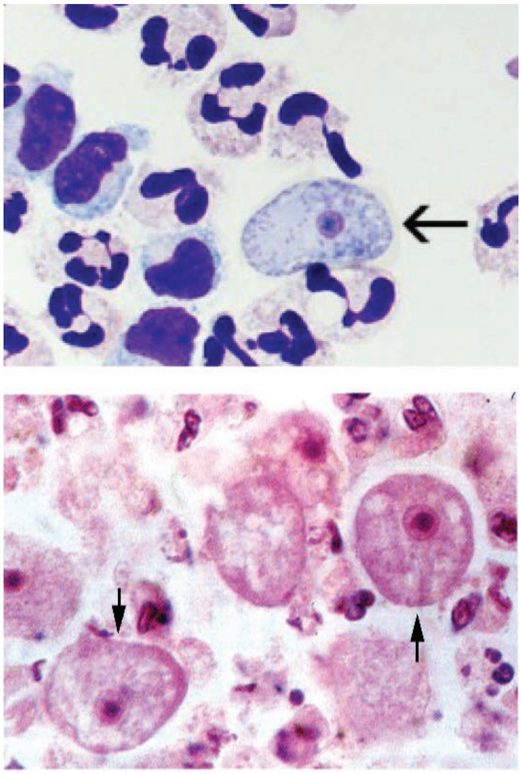


Figure 34.7 *Naegleria fowleri* (primary amebic meningoencephalitis). (Upper) Cytospin of cerebrospinal fluid; note the trophozoite (arrow) with large karyosome (courtesy of the CDC Public Health Image Library). (Lower) *Naegleria* trophozoites in brain tissue; note the large karyosome (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). doi:10.1128/9781555819002.ch34.f7

Balamuthia mandrillaris

Over 200 cases of human amebic encephalitis worldwide have been identified, with >30 being seen within the United States. Death can occur from 1 week to several months after the onset of stroke-like symptoms, which can mimic other conditions including brain stem glioma. Patients eventually die of a massive CNS infection (Fig. 34.8). Both trophozoites and cysts of *B. mandrillaris* are found in many of the same CNS tissues as are *Acanthamoeba* spp. Although differentiation of these two organisms in tissue by light microscopy is difficult, *B. mandrillaris* may have more than one nucleolus in the nucleus in some tissue sections. If the route of entry is through broken skin, painless nodules may be observed which develop into lesions containing trophozoites while the patient is alive. Magnetic resonance imaging (MRI) and computed tomography (CT) are useful and will exhibit brain lesions, which are found in the parietal lobe, anterior lobe, temporal lobe, and cerebellum. Lesions have been described as calcified and as forming a mass-like structure. Biopsy may reveal a necrotic cortex and the faint outline of perivascular monocytes.

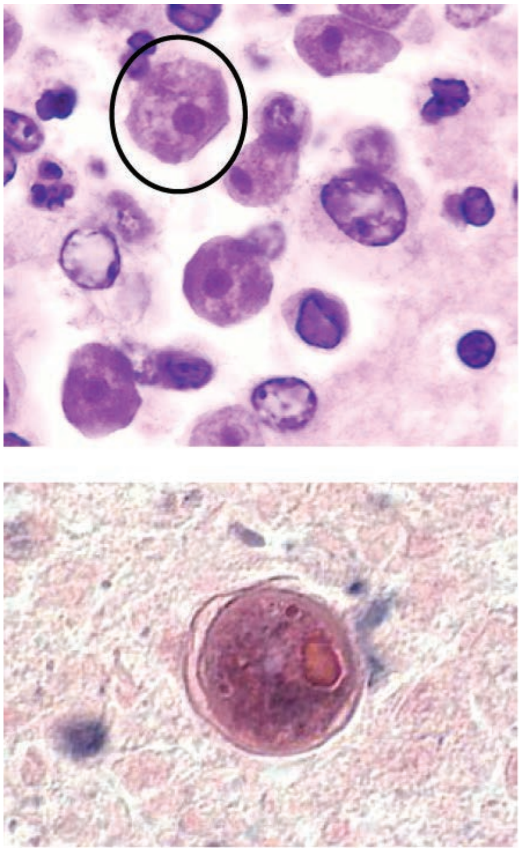


Figure 34.8 *Balamuthia mandrillaris*. (Upper) Trophozoites (circle) are seen in brain tissue. The amebae have the large, characteristic karyosome. The staining of the karyosome is much darker than that of the cytoplasm of the amebae. (Lower) Cyst in brain tissue. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch34.f8

Sappinia pedata

The genus *Sappinia* is a newly recognized human pathogen causing amebic encephalitis; there are two species, *S. diploidia* and *S. pedata* (Fig. 34.9). An infection with *S. pedata* has been well documented in a single patient, who survived the infection, while it is not known whether *S. diploidia* can cause human infections. The patient was a 38-year-old man who complained of visual disturbances and headache; he also had a seizure. He had reported a sinus infection prior to the onset of symptoms. Brain scans revealed a solitary tumor-like mass in the posterior left temporal lobe. On biopsy, the mass demonstrated necrotizing hemorrhagic inflammation, which contained free-living amebae. Histologic findings included central necrotic and hemorrhagic inflammations, acute and chronic inflammatory cells without granulomas or eosinophils, and trophozoites present in viable brain parenchyma on mass periphery without inflammatory response.

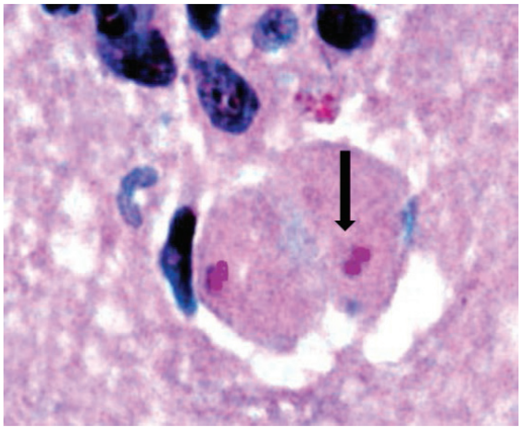


Figure 34.9 *Sappinia pedata*. Trophozoites (arrows) seen in brain tissue; note the presence of two nuclei within some of the trophozoites (arrow) where both are in the same plane of focus (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch34.f9

Flagellates

Giardia lamblia

Trophozoites are detectable in the duodenum and proximal jejunum of infected patients (Fig. 34.10). Although there has been some suggestion of mucosal invasion by this parasite, mucosal invasion has generally been found in areas where necrosis or mechanical trauma was present. In reviewing descriptions of general mucosal appearances, there can be a spectrum of change from normal to almost complete villous atrophy, with a greater density of inflammatory infiltrate in the lamina propria when villous atrophy is present. On histologic slides, trophozoites are most commonly found in duodenal mucosa, with other sites less common (jejunal, gastric antral, and ileal mucosas), but rarely in the colon. An entirely normal light-microscopic appearance of the duodenal mucosa can be found in many patients. Mild villous shortening and mild inflammation of the lamina propria were observed in the duodenal mucosa in some patients. Since the histology of the small-bowel mucosa is inconspicuous in the majority of patients with giardiasis, careful review of biopsy specimens must be performed.

Ciliates

Balantidium coli

Symptoms usually include diarrhea or dysentery, tenesmus, nausea, vomiting, anorexia, and headache. Insomnia, muscular weakness, and weight loss have also been reported. The diarrhea may persist for weeks to months prior to the development of dysentery. There may be tremendous fluid loss, with a type of diarrhea similar to that seen in cholera or in some coccidial or microsporidial infections. Following ingestion, excystation releases trophozoites that invade the colonic mucosa, multiply, and form colonies. *B. coli* propagates within the tissue and may produce ulcers and abscesses that extend to the muscular layer. The symptoms of *B. coli*-induced colitis may be confused with amebic colitis. If extensive damage occurs in the wall of the colon, red blood cells (RBCs) may be seen within the trophozoites, as well as in the stool. *B. coli* produces no known toxins; its ability to produce colonic ulcers is attributed to hyaluronidase, which is an enzyme that digests hyaluronic acid, a component of

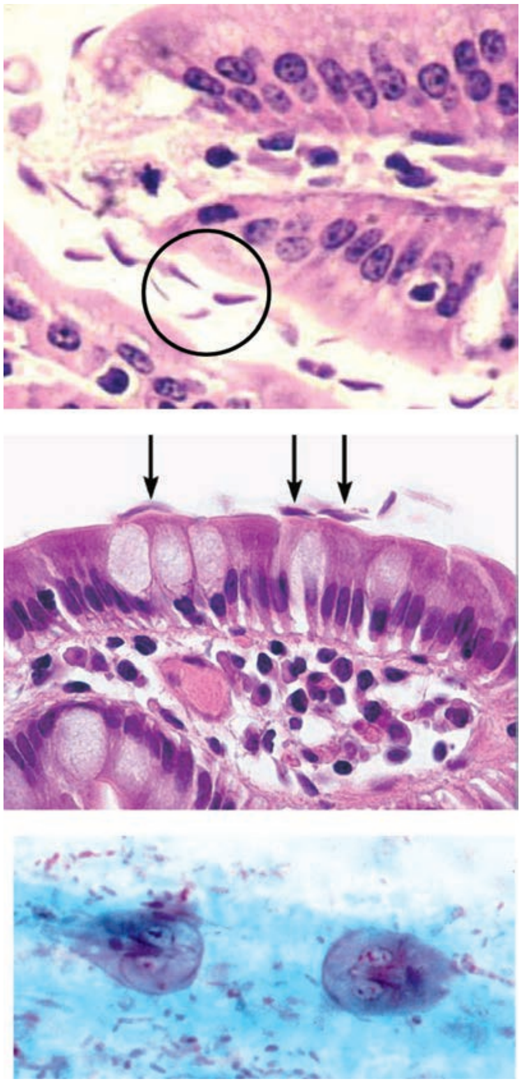


Figure 34.10 *Giardia lamblia* (*duodenalis*, *intestinalis*). (Top) Trophozoites in GI tract (circle). (Middle) Trophozoites on the surface of the duodenal mucosa (arrows) (Armed Forces Institute of Pathology photograph). (Bottom) Trophozoites in mucus from duodenal aspirate, Wheatley's trichrome stain. doi:10.1128/9781555819002.ch34.f10

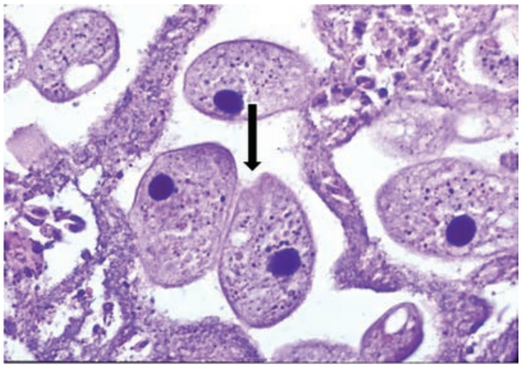


Figure 34.11 *Balantidium coli.* Trophozoites in intestinal tissue; note dark macronucleus and evidence of the oral/feeding groove (arrow). doi:10.1128/9781555819002.ch34.f11

the "glue" holding mucosal epithelial cells together. On contact with the mucosa, *B. coli* may penetrate the mucosa with cellular infiltration in the area of the developing ulcer (Fig. 34.11). Some of the abscess formations may extend to the muscular layer. The ulcers may vary in shape, and the ulcer bed may be full of pus and necrotic debris. Although the number of cases is small, extraintestinal disease has been reported (peritonitis, urinary tract infection, inflammatory vaginitis, pulmonary hemorrhage and respiratory failure, and vertebral osteomyelitis and myelopathy).

Coccidia

Toxoplasma gondii

In immunocompromised patients, the CNS is primarily involved, with diffuse encephalopathy, meningoencephalitis, or cerebral mass lesions. Disseminated toxoplasmosis should be considered in the differential diagnosis of immunocompromised patients with culture-negative sepsis, particularly if combined with neurologic, respiratory, or unexplained skin lesions. It is difficult to demonstrate the parasite in lymph node biopsy specimens, and while some authorities think the histologic appearance is very characteristic, others consider the histologic changes to be nonspecific. However, because many individuals have been exposed to *T. gondii* and may have cysts within the tissues, recovery of organisms from tissue culture or animal inoculation may be misleading, since the organisms may be isolated but may not be the etiologic agent of disease (Fig. 34.12). For this reason, serologic tests are often recommended as the diagnostic approach of choice. However, two representative situations in which the detection of organisms may be very significant are (i) tachyzoite-positive smears and/or tissue cultures inoculated from cerebrospinal fluid and (ii) in patients with acute pulmonary disease, the demonstration of tachyzoites in Giemsa-stained smears of bronchoalveolar lavage fluid, with some tachyzoites being extracellular and some being intracellular.

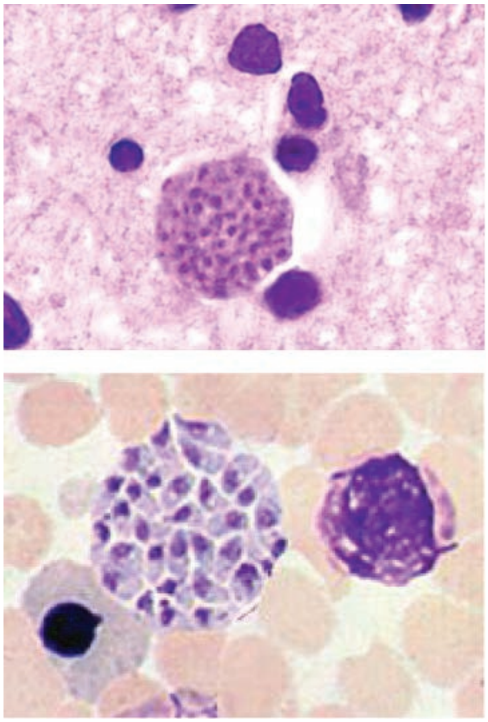


Figure 34.12 *Toxoplasma gondii.* (Upper) Cyst in brain tissue (bradyzoites). (Lower) Tachyzoites in bone marrow. doi:10.1128/9781555819002.ch34.f12

Sarcocystis spp.

Humans can become accidental intermediate hosts for one or more of the 130 known *Sarcocystis* species through ingesting oocysts or sporocysts in food or water contaminated with feces from an infected predator animal. Histopathological differentials include the more common *Toxoplasma* and newer recognized *Neospora* species. Intramuscular infection would be suspected based on symptoms including persistent myalgia, episodic weakness, subcutaneous nodules, dermatomyositis, eosinophilia, and elevated muscle creatinine kinase levels. Often these symptoms, coupled with a history of travel to or living in tropical locations, suggest the next step, which would be a muscle biopsy. Sarcocysts in biopsy specimens can be identified by microscopy of routine histologic sections stained with hematoxylin and eosin (H&E) (Fig. 34.13). Stain variability is common, and periodic acid-Schiff (PAS) positivity of the sarcocyst wall is not always seen. Most sarcocysts in humans have been identified in skeletal and cardiac muscle; however, muscles in the larynx, pharynx, and upper esophagus have also been involved. Sarcocysts are microscopic, up to 700 μm long, and up to 50 μm wide. By light microscopy, the sarcocyst wall is 1 to 2 µm thick. Ultrastructurally, the sarcocyst wall consists of numerous villar protrusions. The villar protrusions are up to 2.8 µm long and 0.4 µm wide, with a tapered end. Microtubules extend from the tip of the villus to the base and occasionally extend deep into the granular layer. The granular layer is approximately 0.5 µm thick. Longitudinally cut bradyzoites were 5.2 by 1.2 (4.8 to 6.5 by 1.0 to 1.3) µm in size (8).

Cryptosporidium spp.

Normal intestinal villi and blunted villi can be seen in Fig. 34.14. Histologically, enteric infections are characterized by villous atrophy, enlarged crypts, and infiltration of the lamina propria by inflammatory cells. Respiratory infections have been characterized by infiltration of the lamina

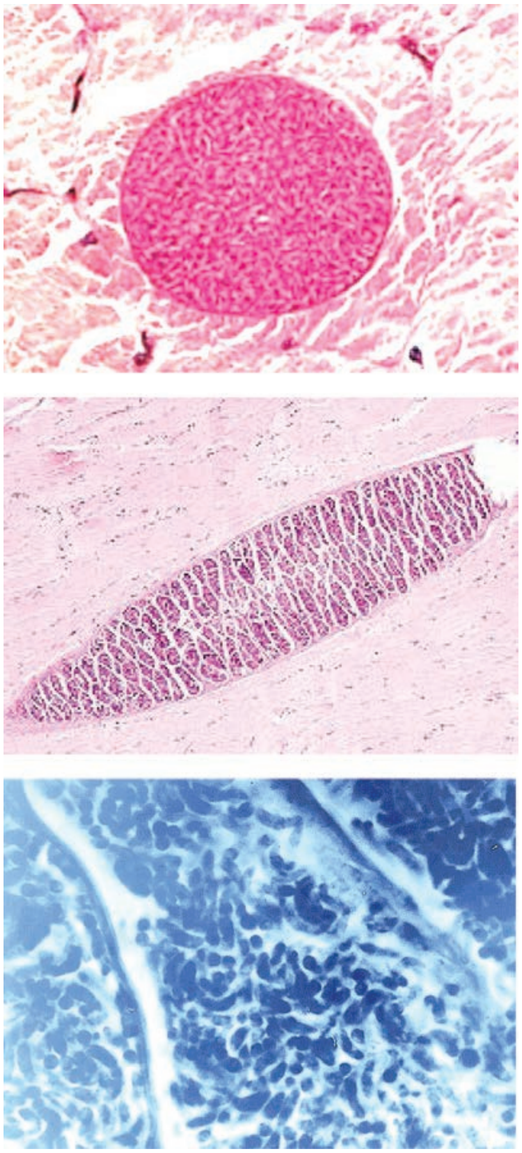
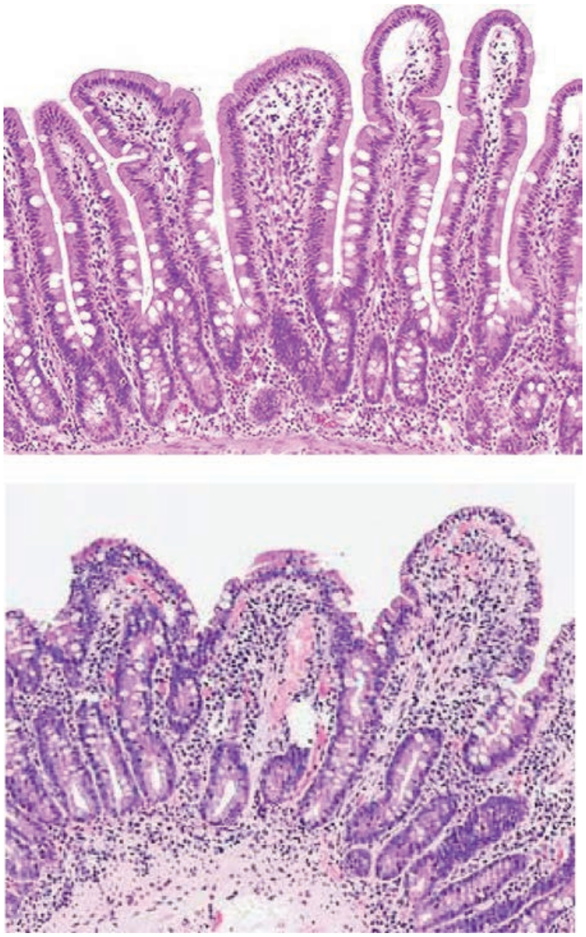


Figure 34.13 *Sarcocystis* spp. (Top) Sarcocyst in tissue. (Middle) Note the septa which are visible in this low-power image. Bradyzoites of *Toxoplasma gondii* in striated muscle resemble *Sarcocystis* spp. However, the *Sarcocystis* bradyzoites are usually more rounded at both ends, and they are contained in larger cysts than those of *Toxoplasma*. (Bottom) The *Sarcocystis* septa are visible, as are the crescentic spores. The sarcocyst wall varies from thin and smooth to thick and striated. Although sarcocysts may be confused with cysts of *Toxoplasma*, sarcocysts tend to be larger and contain larger bradyzoites. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f13

Figure 34.14 Intestinal villi. (Upper) Normal villi are long and slender. (Lower) Blunted villi, typical of histology seen with coccidial infections (*Cryptosporidium* spp., *Cyclospora cayetanensis*) (from reference 14). doi:10.1128/9781555819002.ch34.f14



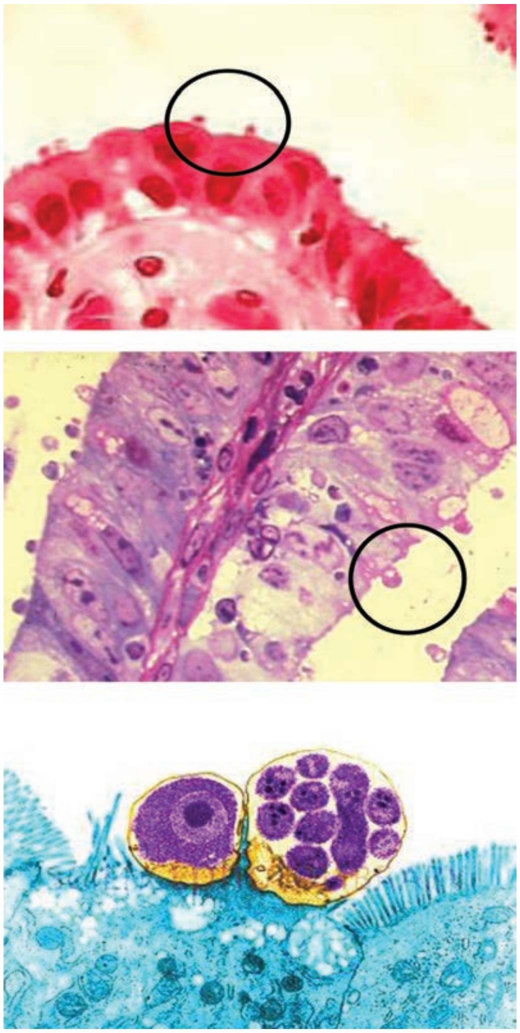


Figure 34.15 *Cryptosporidium* spp. (Top) Organisms shown at the brush border (circle), developing in parasitophorous vacuoles and thus enclosed within membranes of host origin. The mucosal architecture tends to be abnormal, with marked shortening of the villi and hypertrophy of the crypts. (Middle) Organisms shown at the brush border (circle); thick epon section. (Bottom) Color-enhanced transmission electron micrograph showing *Cryptosporidium* (courtesy of the Science Photo Library, http://www.sciencephoto.com/media/445435/view). doi:10.1128/9781555819002.ch34.f15

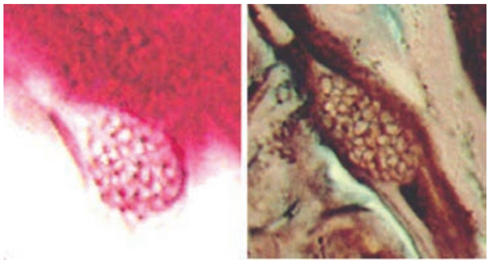
propria by inflammatory cells, loss of cilia, hyperplasia, and hypertrophy of epithelial cells. Similar inflammatory cell infiltration has also been reported for infections involving other organs (Fig. 34.15). Each intracellular stage of *Cryptosporidium* resides within a parasitophorous vacuole within the microvillous region of the host cell. Oocysts undergo sporogony while they are in the host cells and are immediately infective when passed in the stool. Cell damage in enterocyte monolayers has been documented through the disruption of tight cell junctions, a loss of barrier function, the release of lactate dehydrogenase, and increased rates of cell death. Cryptosporidial infection has a predilection for the proximal small bowel, resulting in nonspecific thickening of the duodenum, jejunum, and proximal ileum. Multiple loops of fluid-filled and thickened small bowel loop can be identified on CT.

Microsporidia

It is now well recognized that these unicellular organisms are best described as representing one of many offshoots of the fungal kingdom. Phylogenetic studies have confirmed that the microsporidia evolved from the fungi, being most closely related to the zygomycetes. Other features that are shared with fungi include the presence of chitin and trehalose, similarities in cell cycles, and certain gene organizations. Microsporidia are now considered to be highly derived fungi that underwent genetic and functional losses, thus resulting in one of the smallest eukaryotic genomes known. However, at this point, clinical and diagnostic issues and responsibilities may remain with the parasitologists, and these organisms will be maintained as a part of this parasitology text (Fig. 34.16 to 34.19). Spores are occasionally seen very well by using PAS stain, silver stains, Giemsa stain, or acid-fast stains. Modified Gram stains have also been found to be sensitive although not always standardized. The spore has a small, PAS-positive posterior body, the spore coat stains with silver, and the spores are acid-fast variable. Tissue examination by electron microscopy (EM) is still considered the best approach for identification to the genus/species levels (9–11).

For histologic examination of tissue sections, glutaraldehyde fixation and plastic embedding are highly recommended, even if EM is unavailable. Parasites may be obscured by the host tissue in 6-μm paraffin-embedded preparations.

Figure 34.16 Microsporidia. (Left) Spores in corneal lesion, PAS stain; note the dark-staining dot at the end of each spore. (Right) Spores in corneal lesion, silver stain; note spores appear to be outlined (1,000× oil immersion). Routine histologic testing can be performed by using tissue Gram stains or silver stains. Touch preparations can be methanol fixed and stained with Giemsa stain. Plastic-embedded tissues stained with PAS, silver, acid-fast, and routine H&E stains generally stain better than paraffin-embedded tissues. doi:10.1128/9781555819002.ch34.f16



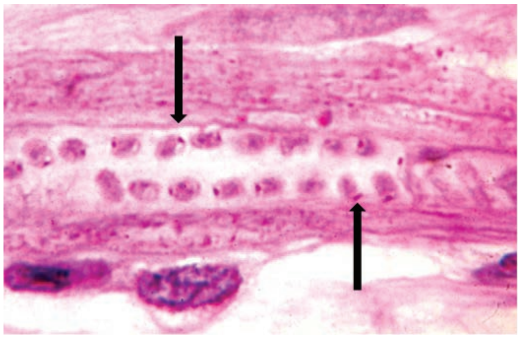


Figure 34.17 Section of appendix showing *Anncaliia connori* spores *in muscularis*. The anterior end of the spore has a PAS-positive granule (arrows) (PAS, ×1,260). (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch34.f17

Using light microscopy, 1-μm-thick plastic sections, heat-fixed to a glass slide and stained with toluidine blue, provide better visualization of microsporidia because of the thinness of the section. Plastic embedding provides a permanent tissue specimen that can be reevaluated by EM at a later time. Additionally, paraffin-embedded tissue can be deparaffinized and reprocessed for plastic embedding.

***Enterocytozoon bieneusi*.** *Enterocytozoon* was the first genus of microsporidia created for a human infection. Primarily, the organism infects the apical portion of enterocytes of the small bowel and produces a slight flattening of the epithelium (chapter 23, Fig. 23.28). Villus atrophy occurs due to rapid desquamation of infected enterocytes. The parasite can disseminate to the epithelial lining of the common bile duct, gallbladder epithelium, and biliary and respiratory tracts. *E. bieneusi* has been documented most often in male AIDS patients, causing malabsorption resulting in chronic diarrhea, and has been associated with AIDS-related sclerosing cholangitis. This microsporidian has also been documented in HIV-negative individuals with and without diarrhea and has been found in tracheal, bronchial, and nasal epithelium.

***Encephalitozoon* spp.** A case of keratoconjunctivitis and chronic sinusitis due to infection with *Encephalitozoon cuniculi* in an AIDS patient has been reported; diagnosis was confirmed by EM. This species has been found in the liver, as well as peritoneal, cerebral, and disseminated infections in AIDS patients. Several eye infections with *Encephalitozoon hellem* have been found in AIDS patients, including the first reported case of *E. hellem* infecting not only sinuses and conjunctivae but also the nasal epithelium. Overall, this species has been documented in disseminated infections and infection of the sinuses, nasal tissue, tongue, respiratory system, kidneys, and male genital tract.

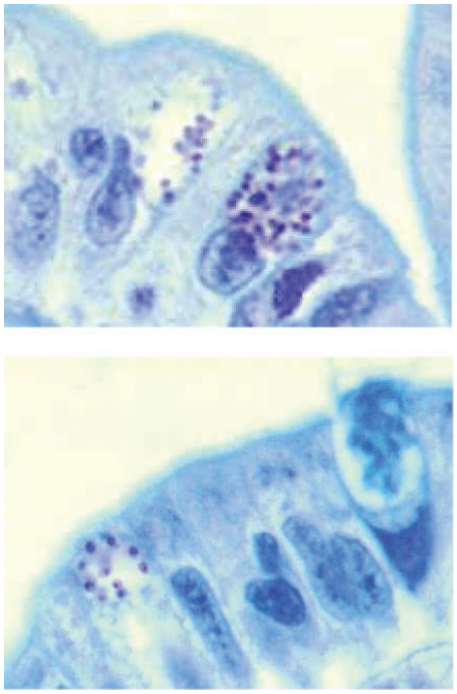


Figure 34.18 Microsporidia in various stages of development in the intestinal enterocytes (1,000× oil immersion). When seen in stool, the spores measure approximately 1 to 3 μm. The microsporidia multiply extensively within the host cell cytoplasm; the life cycle includes repeated divisions by binary fission (merogony) or multiple fission (schizogony) and spore production (sporogony). Both merogony and sporogony can occur in the same cell at the same time. During sporogony, a thick spore wall is formed, providing environmental protection for this infectious stage of the parasite. Microsporidia are characterized by having spores containing a polar tubule, which is an extrusion mechanism for injecting the infective spore contents into host cells. To date, nine genera have been recognized in humans: *Anncaliia*, *Brachiola*, *Encephalitozoon*, *Enterocytozoon*, *Pleistophora*, *Trachipleistophora*, *Vittaforma*, *Microsporidium*, and *Tubulinosema*.
doi:10.1128/9781555819002.ch34.f18

***Encephalitozoon* (*Septata*) *intestinalis*.** *Encephalitozoon (Septata) intestinalis* infects primarily small intestinal enterocytes, but infection does not remain confined to epithelial cells. *E. intestinalis* is also found in lamina propria macrophages, fibroblasts, and endothelial cells. Dissemination to the kidneys, lower airways, and biliary tract appears to occur via infected macrophages. Infections have been reported in the liver, renal system, colon, gallbladder, lungs, sinuses, and conjunctiva.

***Anncaliia* (*Brachiola*, *Nosema*) spp.** *Anncaliia (Brachiola, Nosema) connori* has been identified in human tissues; the spores are oval, measure approximately 2 by 4 μm, and have paired nuclei. The polar tubule has about 11 coils arranged in one to three rows. The single human case

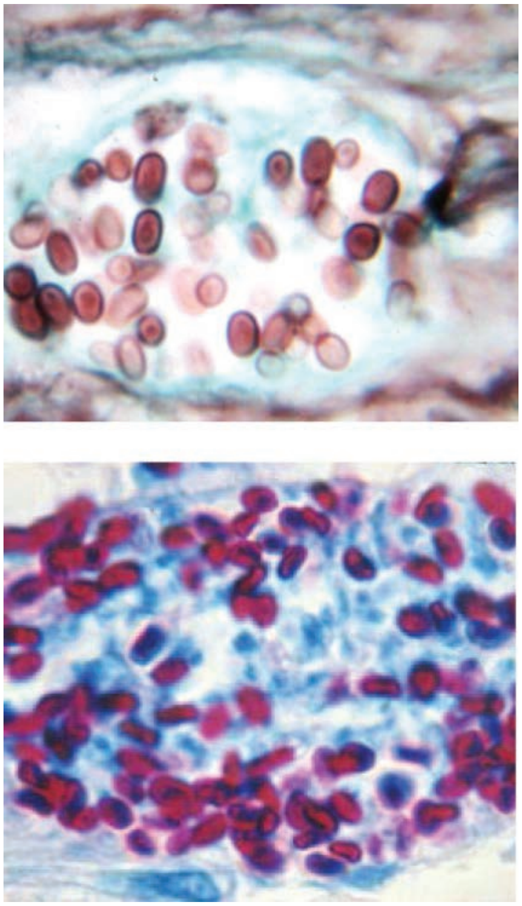


Figure 34.19 (Upper) *Anncaliia connori* spores in wall of ileum; 90-minute Grocott methenamine silver. (Lower) *Anncaliia connori* in myocardium; Ziehl-Neelsen. (Armed Forces Institute of Pathology photographs.) doi:10.1128/9781555819002.ch34.f19

occurred in a 4-month-old infant with combined immunodeficiency disease with a disseminated, fatal infection. Parasites were found in the myocardium, diaphragm, arterial walls, kidney tubules, adrenal cortex, liver, and lungs.

***Anncaliia* (*Brachiola*, *Nosema*) *algerae*.** This microsporidian is apparently transmitted by mosquitoes. Infections have been found in the eye and muscle, possibly from a disseminated case. Both immunocompromised (HIV-negative, but receiving immunosuppressive therapy for rheumatoid arthritis) and immunocompetent patients have become infected. This organism has been known as an insect pathogen for some time. It appears that an initial infection of the eye allows the parasite to adapt to conditions within the mammalian host, thus spreading via macrophages to internal organs.

***Brachiola vesicularum*.** This organism is probably a *Nosema*-like species found in non-HIV-infected patients. Corneal stroma and skeletal muscle were involved in a case in an AIDS patient. Although it was thought that *A. algerae* and *B. vesicularum* might be identical, there are ultrastructural differences between the two organisms. Thus, the designation of two species is currently accepted.

***Microsporidium* spp.** *Microsporidium africanum* was identified in the histiocytes of a corneal ulcer. Although it was first thought to be a *Nosema* sp., electron micrographs confirmed the differences and it was designated *M. africanum*. No information on other hosts is available. *Microsporidium ceylonensis* was found in an eye infection in a child who had been gored by a goat and suffered an eye injury.

***Pleistophora* spp.** Microsporidia of the genus *Pleistophora* have rarely been identified in humans; however, three cases in immunocompetent patients have been reported. In these cases, atrophic and degenerating muscle fibers were full of spores, which were seen in clusters of about 12 organisms, each cluster enclosed by an enveloping membrane, the pansporoblastic membrane. The spores were oval, measuring approximately 2.8 by 3.4 μm. Early biopsies demonstrated atrophic and degenerating muscle fibers; later biopsies showed fibrosis and scarring.

***Trachipleistophora* spp.** *Trachipleistophora hominis* causes severe myositis and sinusitis and was first detected in an AIDS patient. *Trachipleistophora anthropophthera* is the second species in this genus to be described in humans and has been found in brain tissue of two AIDS patients at autopsy; the organisms were also found in the brain, heart, kidney, pancreas, thyroid, parathyroid, liver, bone marrow, lymph nodes, and spleen.

***Vittaforma corneae*.** This organism has been documented in both immunocompromised (urinary tract) and immunocompetent (ocular) patients. An international outbreak of microsporidial keratoconjunctivitis related to soil contact in a Singapore rugby tournament during 2012 has been reported. There were 34/82 (47%) players who were diagnosed with microsporidial keratoconjunctivitis, 3 of which were confirmed as *V. corneae* by PCR (15).

Helminths

Nematodes

See Fig. 34.20 through 34.22 and Table 34.1 for details of the morphology and musculature of the roundworms (Nematodes). There are basically three types of musculature: polymyarian (*Ascaris*), meromyarian (*Enterobius*), and homomyarian (*Trichuris*) (Table 34.1).

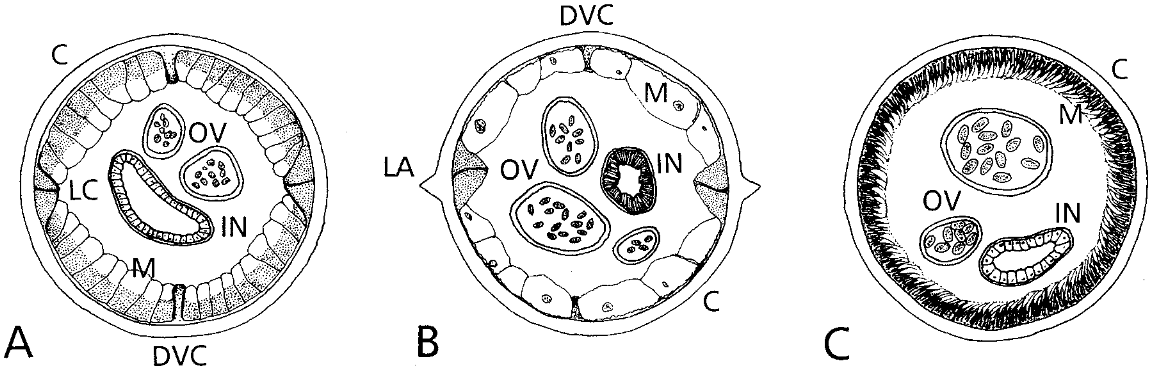
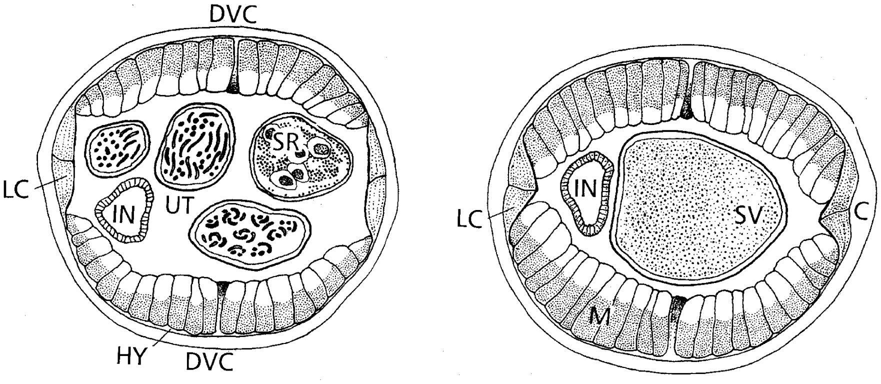


Figure 34.20 Diagram of roundworm musculature morphology in tissue. (A) Polymyarian type. (B) Meromyarian type. (C) Homomyarian type. C, cuticle; DVC, dorsal-ventral chord; IN, intestine; LA, lateral alae; LC, lateral chord; M, muscle; OV, ovary. See Table 34.8. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch34.f20

Figure 34.21 Diagram through *Loa loa* (left, female; right, male) (polymyarian type of musculature). Note the well-developed muscle layer divided into four bands separated by the lateral, ventral, and dorsal chords. The muscle cells project into the body cavity. C, cuticle; DVC, dorsal-ventral chord; HY, hypodermis; IN, intestine; LC, lateral chord; M, muscle; SR, seminal receptacle; SV, seminal vesicle; UT, uterus. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch34.f21



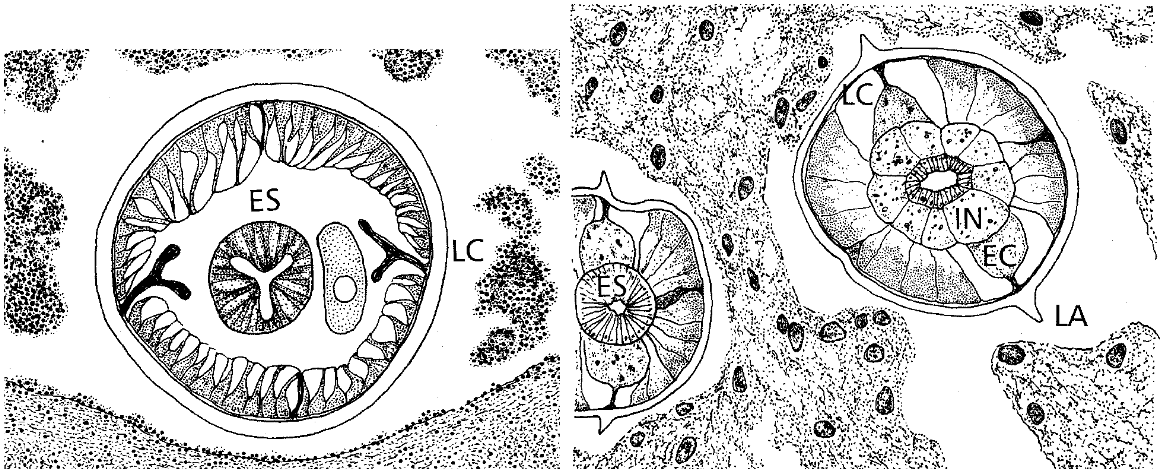


Figure 34.22 Diagram through *Anisakis* (left) and *Baylisascaris procyonis* (right) (polymyarian type of musculature). In *Anisakis*, note the well-developed muscular esophagus (ES) and the Y-shaped lateral chords (LC). In *B. procyonis* (brain tissue), one section is through the esophagus (ES) while the other is through the middle of the body, showing the excretory columns (EC), intestine (IN), and large lateral chords (LC). Note that the lateral alae (LA) are visible in each section. doi:10.1128/9781555819002.ch34.f22

TABLE 34.1 Musculature of nematodes[a]

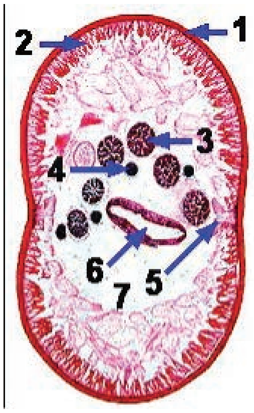
Characteristic	Polymyarian	Meromyarian	Homomyarian
No. of rows of muscle cells	More than 5	Between 2 and 5	Does not exceed 2
Size of cells	Small, uniform	Large, various shapes	Very small, uniform
Arrangement of cells	Projecting into body cavity	Irregular	Regular
No. of cells	Many in each quarter	2 or 3 in each quarter	Many in complete circle
Lateral chords	Present	Present	Absent
Parasites with this type of musculature	*Ascaris*, filaria, *Dracunculus*, *Angiostrongylus*, *Anisakis*	*Enterobius*, hookworms	*Trichuris*, *Trichinella*

[a] Adapted from references 1 to 6.

Ascaris lumbricoides

Although the initial passage of larvae through the liver and lungs usually elicits no symptoms, there can be signs of pneumonitis if the number of larvae is quite large. When the larvae break out of the lung tissue and into the alveoli, there may be some damage to the bronchial epithelium. With reinfection and subsequent larval migrations, there may be intense tissue reactions, even with small numbers of larvae (Fig. 34.23 to 34.25). There may be pronounced tissue reaction around the larvae in the liver and lungs, with infiltration of eosinophils, macrophages, and epithelioid cells. This condition has been called *Ascaris* pneumonitis and is accompanied by an allergic reaction consisting of dyspnea, a dry or productive cough, wheezing or coarse rales, fever (39.9 to 40.0°C), transient eosinophilia, and a chest X ray suggestive of viral pneumonia.

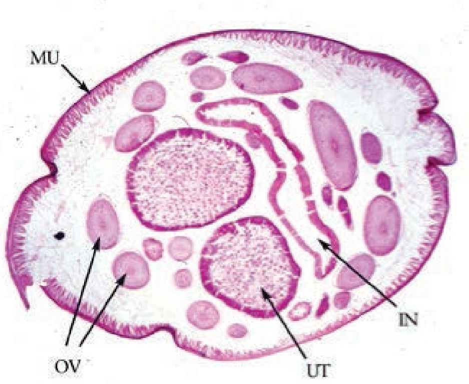
Figure 34.23 Cross section of an adult male *Ascaris lumbricoides*. The section shows the outer protective cuticle secreted by the underlying hypodermis (1). Longitudinal muscle bands (2), dorsal and ventral nerve chords, lateral lines (containing the excretory canals), the intestine, and the pseudocoelom (body cavity) are also visible. The diagram also depicts various sections through the long, coiled testis (4) and vas deferens (3), the latter of which contains the ameboid spermatocytes (sperm cells). Note: These structures may differ in shape, depending on the way they were sectioned. (5) Lateral line with excretory canal; (6) intestine; (7) pseudocoelom. (Courtesy of the Department of Biology, University of Wisconsin-La Crosse, Rick Gillis and Roger J. Haro.) doi:10.1128/9781555819002.ch34.f23



Baylisascaris procyonis

Human infections result from ingestion of eggs that are passed in very large numbers (millions of eggs per day) in the feces of infected raccoons; the human then becomes the accidental intermediate host. Once ingested, the eggs hatch in the intestinal tract, releasing the immature larvae. Rather than developing to adult worms as occurs in the raccoon, the larvae begin to migrate extensively throughout the body tissues, causing visceral larva migrans (VLM) and/or neural larva migrans (NLM). Although this infection presents as acute fulminant eosinophilic meningoencephalitis, two features of the life cycle are somewhat different from those of other helminths causing larva migrans: there is targeted migration to the CNS and continued growth of the larvae to a much larger size within the CNS. Tissue damage is caused by the actual larval migration, as well as an intense inflammatory reaction. Unlike other helminth larvae that cause VLM, the larvae of *B. procyonis*

Figure 34.24 Cross section of an adult female *A. lumbricoides*, stained with H&E. The hypodermis and musculature are visible below the cuticle. Note the presence of the prominent muscle cells (MU), gravid uterus (UT), intestine (IN), and coiled ovary (OV). (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch34.f24



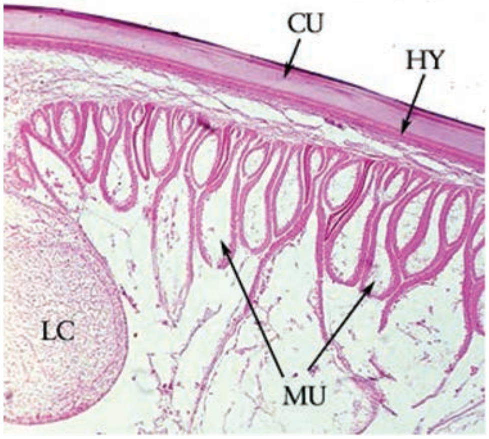


Figure 34.25 Cross section of the cuticle of an adult *A. lumbricoides*, stained with H&E. Shown here are the cuticle (CU) and, immediately below the cuticle, the thin hypodermis (HY). Also shown are the prominent muscle cells (MU) and one of the lateral chords (LC). (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch34.f25

continue to grow during the migratory phase of the life cycle and can reach lengths of 2 mm. In addition to continued growth, the larvae tend to exhibit very vigorous migratory behavior and remain viable for long periods. They tend to invade the eyes, causing ocular larva migrans (OLM), which has also been found in immunocompetent adults, and the spinal cord and brain, causing NLM, found primarily in infants and young children. Unfortunately, the relatively small brain size in infants or small children increases the potential that larval migration will cause severe clinical disease, including permanent neurologic damage, blindness, or death. Numerous granulomata have been seen in the heart, mediastinal soft tissues, pleura and lungs, small and large bowel walls, and mesentery and mesenteric lymph nodes (4). Nonspecific manifestations of this larval migration include a macular rash primarily on the face and trunk, pneumonitis, and hepatomegaly. The development of dyspnea and tachypnea appears to be secondary to early pulmonary migration. The patients may deteriorate rapidly, progressing to stupor, coma, and death. Survivors are left in a persistent vegetative state or with severe neurologic deficits, including blindness, all of which can require extensive supportive care. Cross sections of larvae tend to measure 60 to 70 μm, and the larvae have prominent, single lateral alae and paired, conical excretory columns (smaller than central intestine) (Fig. 34.26).

Lagochilascaris sp.

Lagochilascariasis is an uncommon parasitic nematode infection found in Latin American countries. It is an intestinal parasite of felines like the cloudy leopard, *Felis nebulosa*. As a zoonosis, lagochilascariasis is transmitted to humans, where it is found subcutaneously in unusual locations like tissues of the neck, eye, lungs, mastoid (hard, bony structure behind the ear), and oropharynx (throat at the back of the mouth). The first sign of infection may be a pustule, usually on the neck, which increases in size to a large swelling. After the skin breaks, living adults, larvae, and eggs are expelled at intervals in the pus. The area of induration increases, with the development of sinus tracts. Chronic granulomatous inflammation may last for months. In addition to humans, it has been found to parasitize humans, domestic cats, dogs, and sylvatic, or forest-dwelling, carnivores (Fig. 34.27).

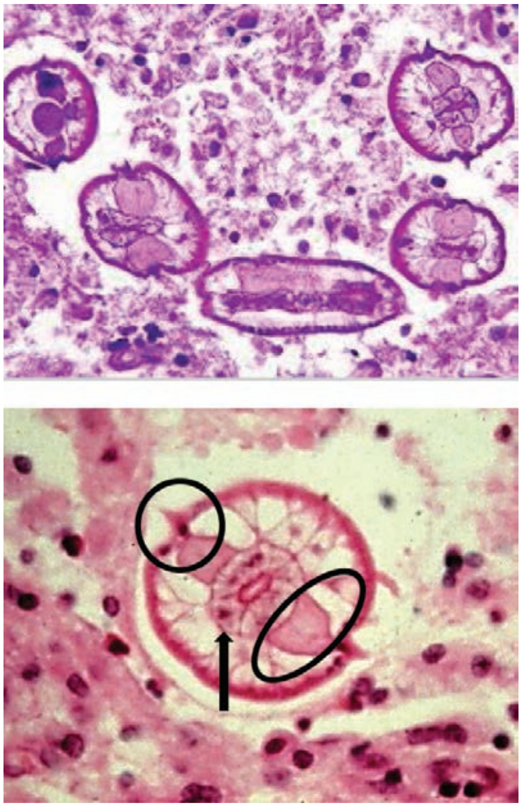


Figure 34.26 *Baylisascaris procyonis*. (Upper) CNS atrophy, sclerosis, and larval granuloma in the brain in a 2-year-old boy who died following 14 months of CNS problems. (Lower) *B. procyonis* larva (polymyarian type of musculature) and inflammation in the corpus callosum of an infected rabbit. Note the prominent alae (circle), excretory columns (oval), and multinuclear intestinal cells (arrow). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of K. R. Kazacos.) (See also Fig. 34.22.) doi:10.1128/9781555819002.ch34.f26

Toxocara spp.

VLM Syndrome. The VLM syndrome is most commonly seen in children 1 to 4 years of age; however, rare cases have been seen in adults, in whom the illness includes mild pulmonary involvement suggestive of VLM. The full clinical presentation includes hepatomegaly and pulmo-

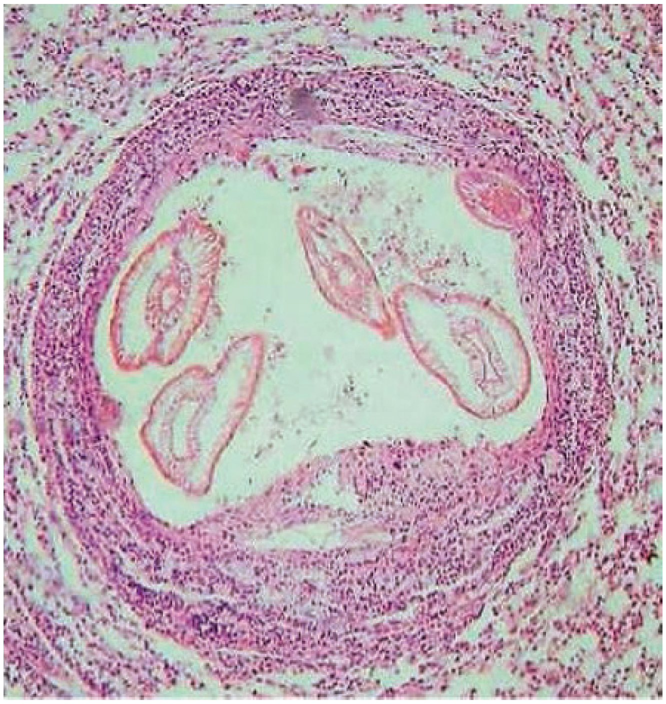


Figure 34.27 *Lagochilascaris* (polymyarian type of musculature) encapsulated larvae in mouse lung tissue with varying degrees of damage, including secondary granuloma containing larvae in different degrees of destruction, concentric fibrosis, and foci of inflammatory infiltration composed of macrophages (H&E). (Courtesy of U.S. National Library of Medicine, National Institutes of Health.) doi:10.1128/9781555819002.ch34.f27

nary infiltrates or nodules accompanied by cough, wheezing, eosinophilia, lymphadenopathy, and fever. Larval entry into the CNS can also result in meningoencephalitis and cerebritis manifesting as seizures. Larvae often remain in the liver and/or lungs, where they become encapsulated in dense fibrous tissue. Other larvae may continue to migrate throughout the body, causing inflammation and granuloma formation.

OLM Syndrome. Evidence suggests that ocular disease can occur in the absence of systemic involvement and vice versa for VLM. Although these facts may be explained by possible strain differences of *Toxocara* spp., VLM may reflect the consequences of the host inflammatory response to waves of migrating larvae while OLM may occur in individuals who have not become sensitized. The resulting inflammation presents clinically as either a granuloma or a granulomatous larval track in the retina or as a condition of the vitreous that resembles endophthalmitis (Fig. 34.28).

Anisakis (Larval Nematodes Acquired from Saltwater Fish)

The majority of the cases of anisakiasis involve gastric anisakiasis. Colonic anisakiasis is extremely rare; fewer than 100 cases have been reported from the United States. However, this infection is probably misdiagnosed and underreported. Human infection is acquired by the ingestion of raw, pickled, salted, or smoked saltwater fish or squid (Fig. 34.29). The larvae often penetrate into the walls of the digestive tract (frequently the stomach), where they become embedded in eosinophilic granulomas. Occasionally, the throat is involved. These large larvae (third stage) measure 1 to 3 cm long or more by 1 mm wide. Histologic sections are characterized by the large body size, moderately thick cuticle, and large lateral cords that extend into the body cavity.

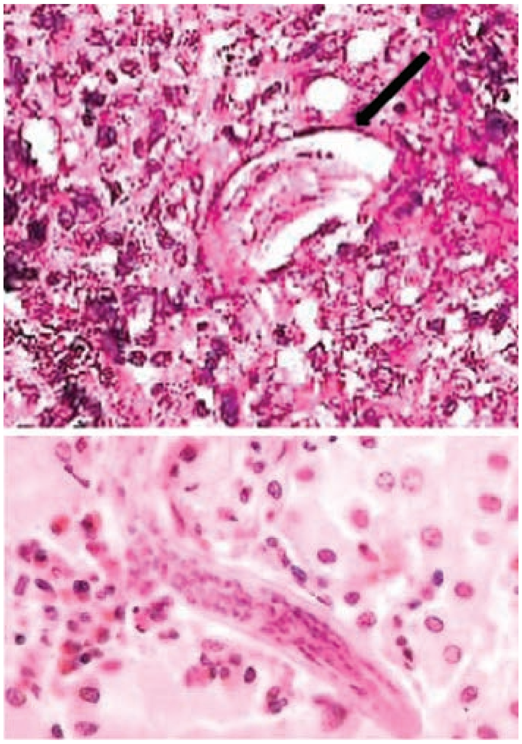


Figure 34.28 *Toxocara*. (Upper) *Toxocara* larva present in the eye (arrow). Eosinophil-rich vitreous abscess contains a fragment of a nematode larva (H&E). (Lower) *Toxocara* larva recovered in liver (visceral larva migrans). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f28

Dirofilaria spp.

Human dirofilariasis is typically seen as subcutaneous nodules or as lung parenchyma disease. Patients infected with *Dirofilaria repens* tend to have a subcutaneous lump (face and conjunctiva of the eye and sometimes chest wall, upper arms, thighs, abdominal wall, and male genitalia) (Fig. 34.30). Ocular involvement is usually periorbital, orbital, subconjunctival, or subtenon infection. Human *Dirofilaria immitis* infection is associated with human pulmonary dirofilariasis and is usually asymptomatic. If symptoms are

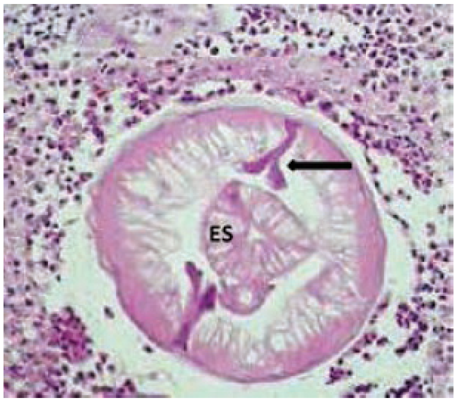


Figure 34.29 *Anisakis* cross section. Note the typical polymyarian type of musculature and the Y-shaped lateral chords (arrow). The large esophagus is in the center of the image (ES). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) (See also Fig. 34.22.) doi:10.1128/9781555819002.ch34.f29

present, they include cough, chest pain, fever, and pleural effusion. When the worms lodge in the pulmonary artery branches, they cause an infarct. These lesions are usually on the periphery of the lungs and are sharply defined (coin lesion). There is a central necrotic area surrounded by a granulomatous inflammation and a fibrous wall. Dead or dying worms may be found in the lesion. In all cases, histology reveals a histiocyte-rimmed necrotic nodule containing fragments of a partially degenerated *D. immitis* worm, while adjacent lung parenchyma shows morphological features consistent with other pulmonary processes such as extrinsic allergic alveolitis and/or pulmonary vasculitis syndromes. Ocular lesions are usually associated with moderate to severe inflammation. Most cases with eye infections present with pain in the eye, redness, sometimes blurred vision, and swelling of eyelids.

Because the immature larvae may be detected in only a few microscopic sections, careful histologic examination is necessary. The cuticle of nematodes contains chitin, which can be stained with nonspecific whiteners such as calcofluor white. *Dirofilaria* larvae stained with calcofluor white can be easily recognized in tissue sections, whereas the parasite may be difficult to identify using routine histologic stains.

Capillaria hepatica

Capillaria hepatica infection is commonly found in rats, other rodents, and other mammals. Human cases have been reported from various parts of the world, including the United States. Infection occurs via accidental ingestion of eggs from the soil. These eggs hatch and are carried via the portal system to the liver, where the larvae mature in approximately 4 weeks and begin to deposit eggs in the liver parenchyma (Fig. 34.31). Symptoms of this infection

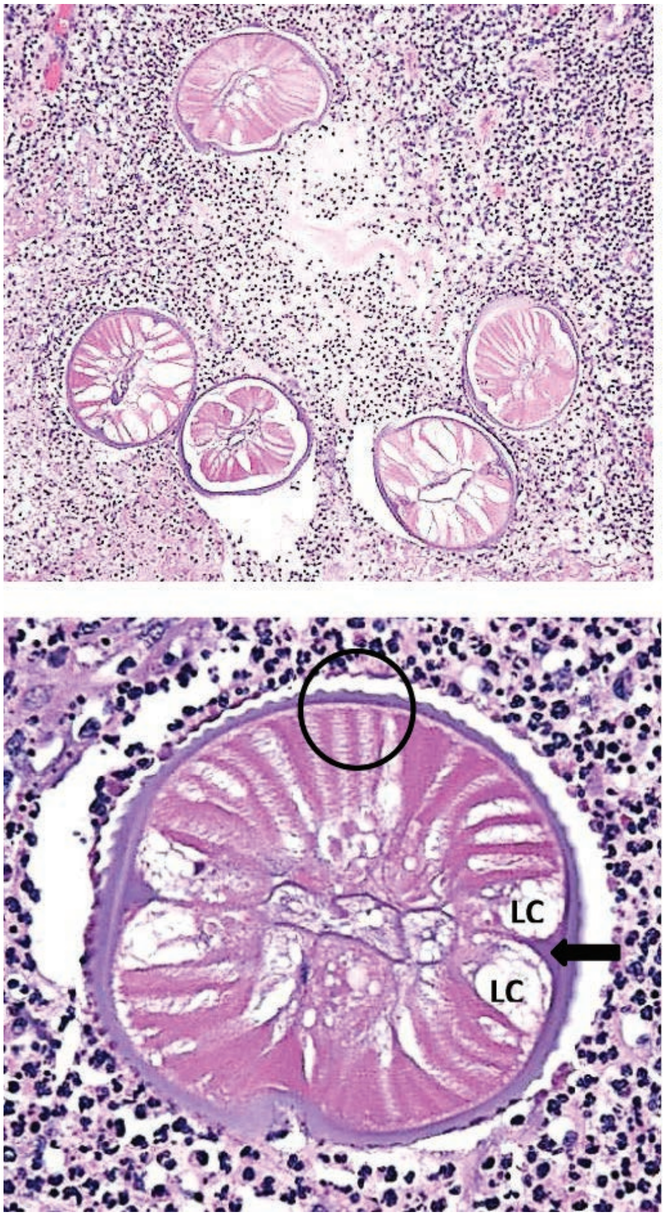


Figure 34.30 *Dirofilaria* sp. (Upper) Note the polymyarian type of musculature and the clearly delineated lateral chords. Also note the longitudinal ridges in the cuticle (bumpy appearance). (Lower) Higher magnification than upper image. Note the typical polymyarian type of musculature and the more clearly delineated longitudinal ridges on the inner surface of the cuticle (circle). The cuticle is relatively thick and multilayered. The very prominent lateral chords are also visible (LC), as is the lateral ridge (arrow); although ventral and dorsal chords are present, they are usually inconspicuous. (Courtesy of Marjorie R. Fowler and Andrea Linscott.) doi:10.1128/9781555819002.ch34.f30

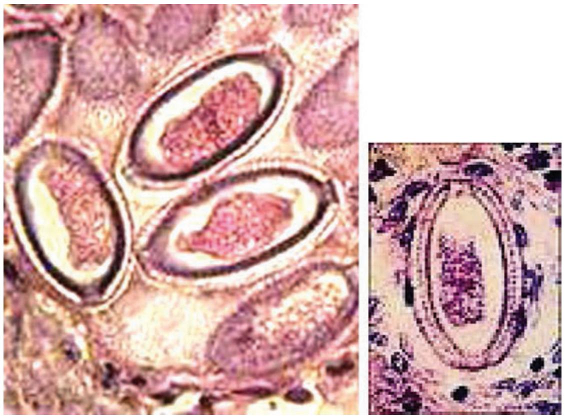


Figure 34.31 *Capillaria hepatica* eggs in liver. These eggs resemble those of *Trichuris trichiura*, but the shells in *Capillaria* eggs are striated. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch34.f31

mimic those of hepatitis, amebic liver abscess, trichinosis, VLM, Loeffler's syndrome, Hodgkin's disease, and histoplasmosis. In a true human infection, no eggs are found in the stool. Diagnosis requires histologic examination. Eggs in liver biopsy specimens can be identified on the basis of their characteristic morphology.

Enterobius vermicularis

Although enterobiasis is an uncommon cause of acute appendicitis in children within the United States, this infection may be associated with acute appendicitis, chronic appendicitis, or ruptured appendicitis, or with no significant symptoms (Fig. 34.32 to 34.34). Other uncommon ectopic sites have included the peritoneal cavity, lungs, liver, urinary tract, and natal cleft. Pathologic examination usually reveals chronic granulomatous inflammation with or without central necrosis, which is surrounded by polymorphonuclear neutrophilic leukocytes, eosinophils, and fibroblasts. Macrophages, giant cells, epithelioid cells, and Charcot-Leyden crystals may also be present. During a case of suspected transverse colon carcinoma, histologic examination of the mass revealed eggs of *E. vermicularis* embedded in granulomatous tissue in the submucosa of the colon; no malignancy was found.

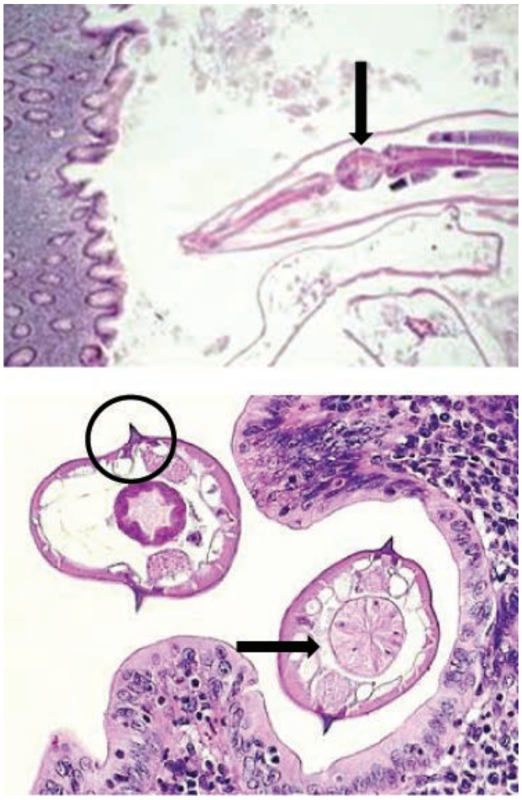


Figure 34.33 (Upper) Longitudinal section of female *Enterobius vermicularis* in the cecum, H&E stain; note the esophageal bulb (arrow) (Korea Society for Parasitology, Sung-Jong Hong). (Lower) Intestinal lumen containing two transverse sections of an *Enterobius vermicularis* worm. This nematode has the meromyarian type of musculature. Note the lateral alae (circle) and intestine (arrow). (Armed Forces Institute of Pathology photograph.)
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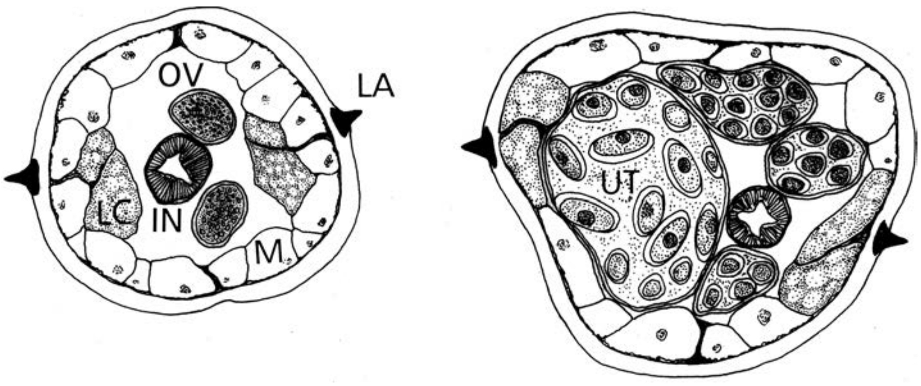

Figure 34.32 Diagram through *Enterobius vermicularis* (both images are of females) (meromyarian type of musculature). Note the very conspicuous lateral alae (LA). The muscle cells (M) are large, of various shapes, and irregular; there are fewer cells per quadrant than seen in the polymyarian type (Fig. 34.20). The lateral chords (LC), intestine (IN), ovary (OV), and uterus (UT) are also visible. (Illustration by Sharon Belkin.)
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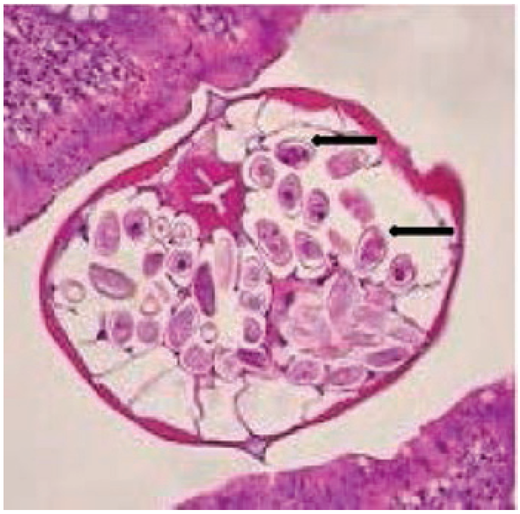

Figure 34.34 Cross section of adult *Enterobius vermicularis* female worm in the appendix. Note the characteristic football-shaped eggs (arrows). The lateral alae are also visible. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
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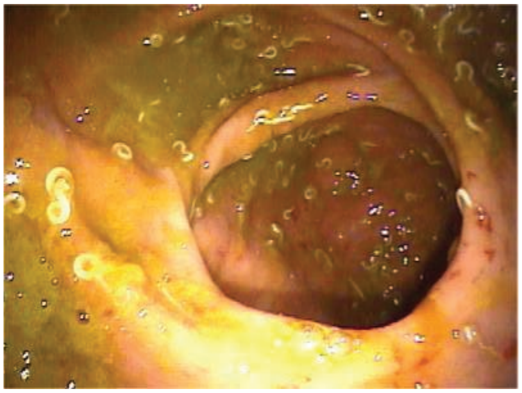

Figure 34.36 Colon heavily infected by *Trichuris trichiura*; photo taken during colonoscopy. (Courtesy of American Association for the Advancement of Science; reference 12.)
doi:10.1128/9781555819002.ch34.f36

Trichuris trichiura

The adult worms are rarely recovered from the stool, since they are attached to the wall of the intestine. The head portion of the worm is very thin and is embedded in the mucosa, while the posterior end is much thicker and lies free in the lumen of the large intestine (Fig. 34.35 to 34.37). The large, posterior end has been described as the "whip handle" while the thin, anterior end is the "whip" itself, hence the name whipworm. The differential diagnosis of chronic diarrhea requires consideration of several diseases, including celiac disease, inflammatory bowel disease, and irritable bowel syndrome (12). Patients infected with *T. trichiura* may present with a chronic dysentery-like syndrome if they have a massive infestation, leading to anemia and growth retardation. In some cases, diarrhea may last for years without blood and mucus. However, once blood is evident, medical intervention may occur; in some cases the diagnosis requires colonoscopy. Mechanical damage to the mucosa and the allergic response by the host appear to be the main reasons for any abnormalities associated with *T. trichiura* infection and are definitely related to the worm burden, the length of the infection, and the age and overall health status of the host. Although the worm is actually threaded into the epithelium of the cecum, damage produced by this process is minimal unless there is dysen-

Figure 34.35 Diagram through *Trichuris trichiura* (female on the left, male on the right) (homomyarian type of musculature). Note the very small, uniform muscle cells (M) with a regular arrangement, with many cells arranged in a complete circle. Note also the absence of lateral chords. The ejaculatory duct (EJ), intestine (IN), ovary (OV), oviduct (OVD), uterus (UT), and testis (TE) can also be seen. (Illustration by Sharon Belkin.)
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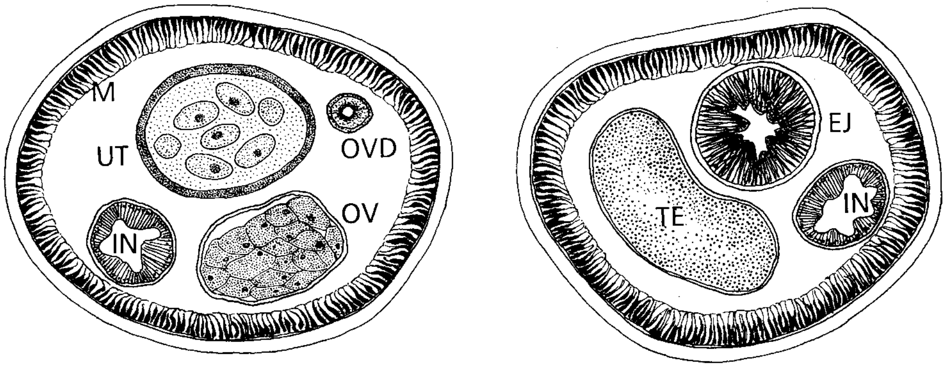


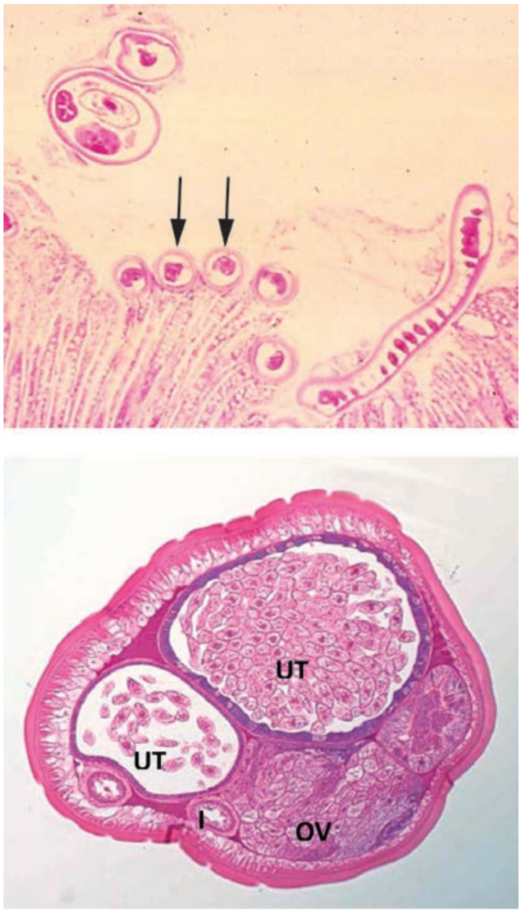


Figure 34.37 *Trichuris trichiura*. (Upper) Several sections of adult male and female *T. trichiura* worms in the colon, showing the narrow anterior portion of the worm within the mucosa ("whip") (arrows) and the thicker posterior portion ("handle") free in the lumen (×15; Armed Forces Institute of Pathology photograph). (Lower) Cross section of female worm (I, intestine; OV, ovary; UT, uterus). The musculature is of the homomyarian type, with very small, uniform, regular cells with many cells in a complete circle. No lateral chords are present. (Courtesy of Andrea Linscott.) doi:10.1128/9781555819002.ch34.f37

tery, during which the mucosa may be edematous and friable, and there may be abdominal cramps, severe rectal tenesmus, and rectal prolapse. The dysentery seen in severe infections may mimic that seen in amebiasis.

Hookworms (*Ancylostoma*, *Necator*)

Adult worms are rarely seen, since they remain firmly attached to the intestinal mucosa by means of well-developed mouth parts (teeth in *Ancylostoma duodenale* and cutting plates in *Necator americanus*) (Fig. 34.38 and 34.39). Symptoms from the intestinal phase of the infection are caused by (i) necrosis of the intestinal tissue within the adult worm mouth and (ii) blood loss by direct ingestion of blood by the worms and continued blood loss from the original attachment site, possibly as a result of anticoagulant secreted by the worm.

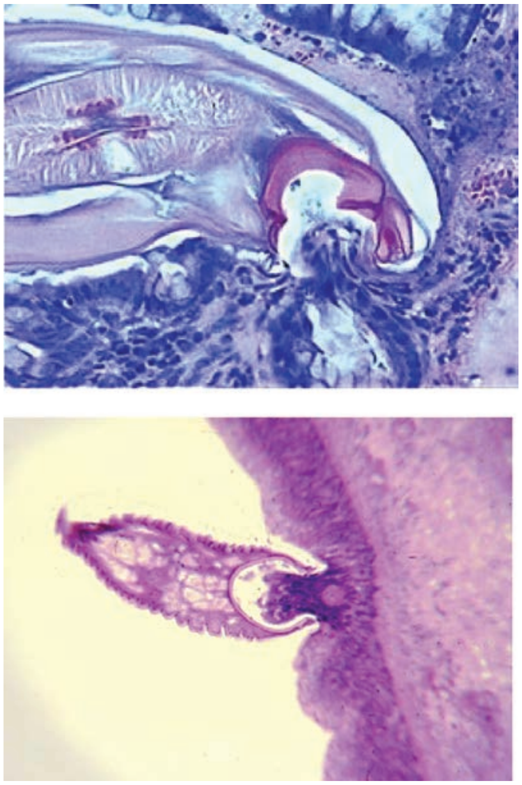


Figure 34.38 *Ancylostoma caninum*. (Upper) Adult hookworm attached to the intestine (courtesy of Richard Bungiro, Yale University). (Lower) Hookworm; section of a worm attached to the mucosa. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f38

Trichinella sp.

Human infection is initiated by the ingestion of raw or poorly cooked pork, bear, walrus, or horse meat or meat from other mammals (carnivores and omnivores) containing viable, infective larvae. The very active muscles including the diaphragm, muscles of the larynx, tongue, jaws, neck, and ribs, the biceps, and the gastrocnemius, which have the greatest blood supply, are invaded. The encysted larvae may remain viable for many years, although calcification can occur within less than a year. As few as 5 larvae/g of body muscle can cause death, although 1,000 larvae/g have been recovered from individuals who died from causes other than trichinosis. Pathologic changes due to trichinosis can be classified as (i) intestinal effects and (ii) muscle penetration and larva encapsulation.

During muscle invasion, there may be fever, facial (particularly periorbital) edema, and muscle pain, swelling, and weakness. Other signs are conjunctivitis, headache,

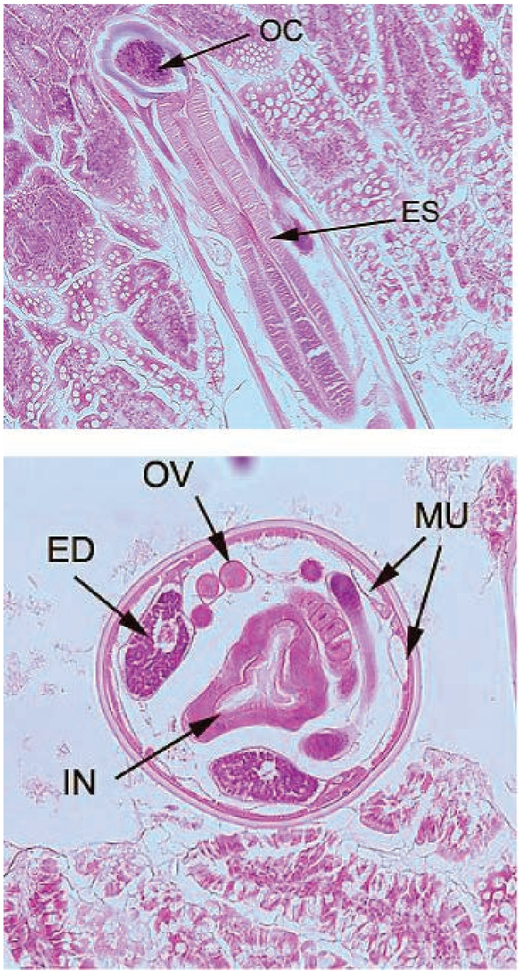


Figure 34.39 Hookworm. (Upper) Longitudinal section of an adult hookworm worm in a bowel biopsy, stained with H&E. Note the oral cavity (OC) and strong, muscled esophagus (ES). (Lower) Cross section of an adult hookworm from the same specimen in upper image. Shown here are the musculature (MU), intestine with brush border (IN), excretory ducts (ED), and coiled ovaries (OV). (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch34.f39

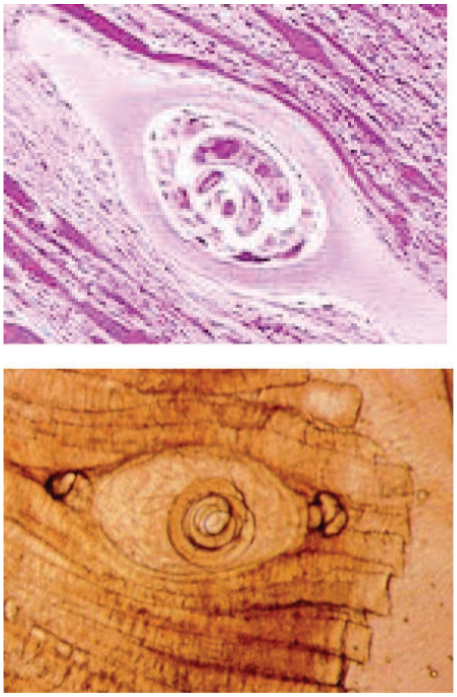
dry cough, petechial bleedings, and painful movement disorder of the eye muscles. The extraocular muscles are usually the first to be involved, followed by the muscles of the jaw and neck, limb flexors, and back. Muscle damage may cause problems in chewing, swallowing, breathing, etc., depending on which muscles are involved. The most severe symptom is myocarditis, which occurs in approximately 5 to 20% of cases and which usually develops after the third week. Symptoms include pericardial pain, tachycardia, and electrocardiogram abnormalities such as nonspecific ventricular repolarization disturbances, followed by bundle-branch conduction disturbances, and sinus tachycardia. Reversal of the potassium deficit corrects the electrocardiogram abnormalities. Screening should be performed for all patients suspected of having trichinosis; serum troponin provides a simple and reliable means and can be positive even in asymptomatic myocarditis.

It is estimated that 10 to 20% of the patients with trichinosis have CNS involvement and that the mortality rate may reach 50% in these patients if they are not treated. Symptoms may mimic those of polyneuritis, acute anterior poliomyelitis, myasthenia gravis, meningitis, encephalitis, dermatomyositis, and polyarteritis nodosa. There may be focal paresis or paralysis (quadriplegia to single muscle group).

Peripheral eosinophilia of at least 20%, often over 50%, and possibly up to 90% is present during the muscle invasion phase of the infection. Fever can also be present at this time and can persist for several days to weeks, depending on the intensity of the infection. However, once the larvae begin to encapsulate, patient symptoms subside; eventually the cyst wall and larvae calcify (Fig. 34.40).

Muscle biopsy (gastrocnemius, deltoid, and biceps) specimens may be examined by compressing the tissue between two slides and checking the preparation under a microscope at low power (10× objective). However, this

Figure 34.40 *Trichinella spiralis*. (Upper) Encysted larvae in muscle. Although *Trichinella* spp. have the homomyarian type of musculature, the magnification of this image is insufficient to show the details. (Lower) Squash preparation of tissue biopsy specimen. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f40



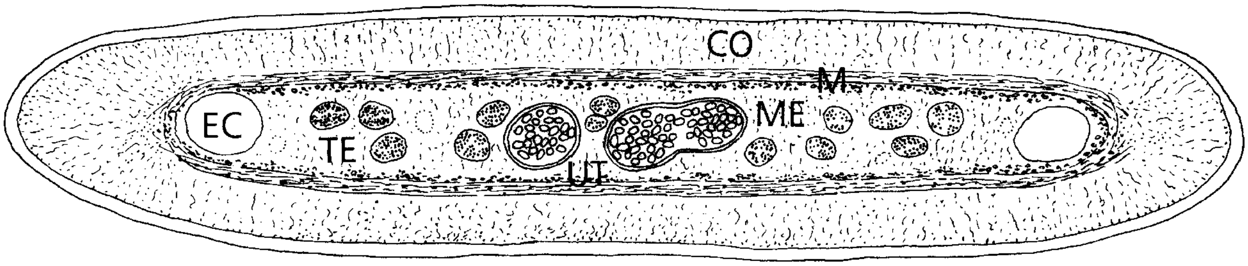


Figure 34.41 Diagram of a transverse section of a *Taenia* proglottid. Under the thick tegument, the underlying parenchyma is divided into cortical (CO) and medullary (ME) layers by a thick band of longitudinal muscles (M). The excretory columns (EC) are clearly visible. Branches of the uterus (UT) and testes (TE) are also seen. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch34.f41

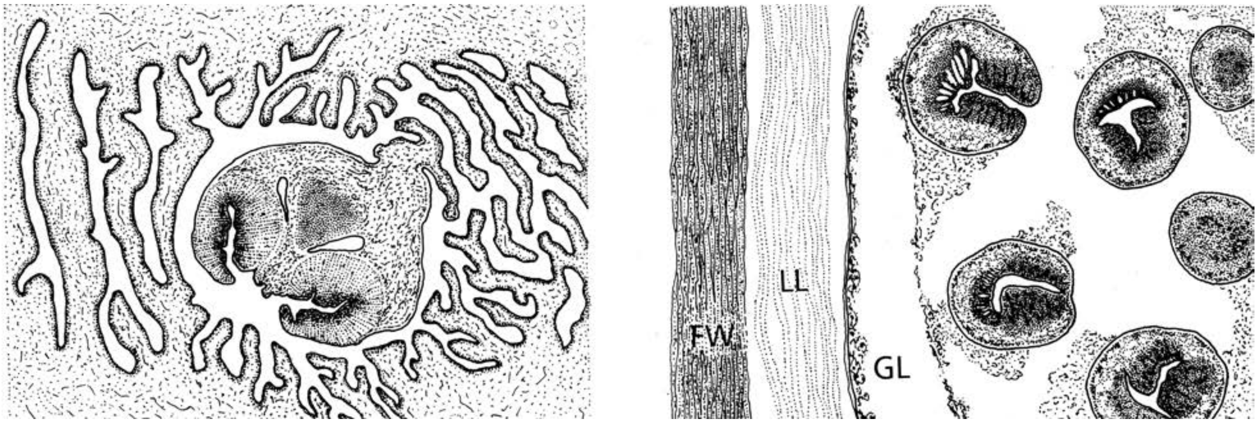
method does not provide positive results until 2 to 3 weeks after the onset of the illness. It is also important to remember that not all species form the capsule.

Cestodes

Taenia solium Cysticercosis, Racemose Cysticercosis

Cysticercosis. Cysticerci have been recovered from all areas of the body, and symptoms depend on the particular body site involved (Fig. 34.41 to 34.45). The presence of cysticerci in the brain represents the most frequent parasitic infection of the human nervous system and the most common cause of adult-onset epilepsy throughout the world. For unknown reasons, in Latin America it is unusual for both brain and muscle cysticercosis to occur in the same patient, with fewer than 6% of patients having cysticerci in both sites. However, elsewhere in the world, subcutaneous involvement by *T. solium* cysticerci has been detected in as many as 78.5% of patients with cerebral cysticercosis (4). Possible reasons for such differences may include (i) the immune status of the patient, (ii) the human leukocyte antigen type, (iii) the nutritional status of the patient, (iv) the burden of eggs infecting the patient, and (v) a difference in the strains of *T. solium*. It is also rare for a patient with cysticercosis to harbor adult *T. solium* worms in the intestine. Neurocysticercosis usually affects males and females of all ages, with a peak incidence between 30 and 50 years of age. It has been estimated that 50,000 individuals die annually due to neurocysticercosis.

Figure 34.42 (Left) Diagram of a cysticercus of *Taenia solium*. The extensively folded spiral canal and a scolex with two large suckers are clearly visible. The denser tissue of the rostellum (between the two suckers) is also visible. In this particular section plane, the hooklets are not visible. Host tissue forms a fibrous capsule around the cysticercus. (Right) Diagram of a unilocular hydatid cyst of *Echinococcus granulosus*. The outer thick fibrous wall is visible (FW) and is produced by the host. Within the fibrous layer is the thinner laminated layer (LL), while right below that layer is the thin germinal epithelial layer (GL) from which daughter cysts and multiple protoscolices arise. Multiple protoscolices within a brood capsule are visible in this illustration. Note that the middle laminated layer is acellular and the thin germinal layer contains some calcareous corpuscles. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch34.f42



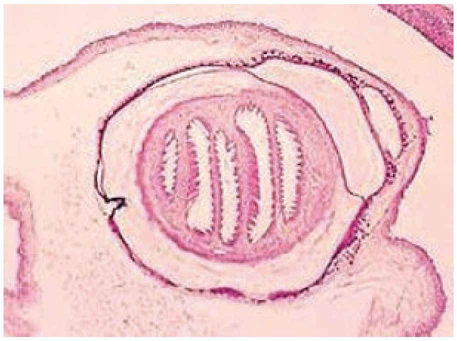


Figure 34.43 *Taenia solium* cysticercus in pig muscle. The cysticercus consists of a fluid-filled bladder containing a single protoscolex. The cysticerci (*T. solium*) have a thick bladder wall, and the rostellum has two rows of hooklets (13 each). Each cysticercus measures 5 to 15 mm long by 4 to 12 mm wide. A convoluted spiral canal leads to the rostellum. The parenchyma usually contains calcareous corpuscles. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of P. M. Schantz.)
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Neurocysticercosis is the most common parasitic disease of the CNS; in some countries the prevalence exceeds 10% and is responsible for up to 50% of late-onset epilepsy cases. Although parenchymal cysts are the most common location in the brain, cysts may also be present in the ventricles, meninges, spinal cord, and subarachnoid spaces. Cysticerci have been recovered from the brain (all areas), spinal cord, breast, thyroid, eye (orbit, subretinal), muscles (various sites), mouth, salivary gland, tongue, lip, masseter muscle, pharynx, nose, kidney, lymph nodes, pituitary gland, hypothalamus, parotid gland, submandibular, thorax, scrotum, heart, and subcutaneous tissue, and from disseminated infections.

Cysticerci are milky white, spherical to oval, and usually about 1 cm when developed. Each cyst contains fluid and a single invaginated protoscolex, which has four large spherical suckers and a rostellum armed with a double row of 22 to 36 large and small hooklets. Hooklets are large, readily visible in stained tissue sections, and birefringent and may be acid fast. The wall of the bladder is 100 to 200 µm thick and consists of a tegument, muscle cells, tegumental cells, and parenchyma. The bladder tegument is thin, bears microvilli on the outer surface, and is frequently raised into projections 10 to 25 µm wide. Smooth muscle fibers lie beneath the tegument and extend into the parenchyma, with a row of tegumental cells lying below the muscle fibers. The parenchyma consists of loose stroma, fluid-filled spaces, calcareous bodies, mesenchymal fibers, and smooth muscle.

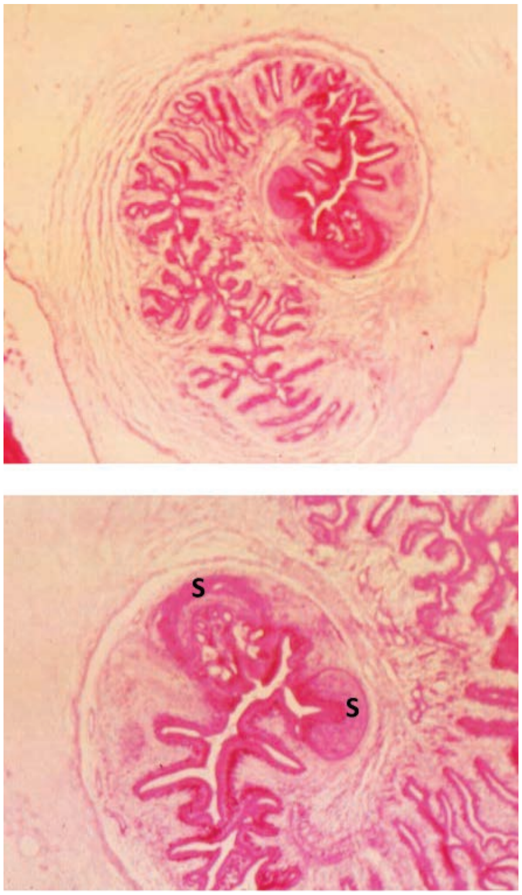

Figure 34.44 *Taenia solium.* (Upper) Subcutaneous nodule from a patient. (Lower) Higher magnification than upper figure; note the suckers (S). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of K. Juniper, Jr.)
doi:10.1128/9781555819002.ch34.f44

Racemose Cysticercosis. The racemose form consists of several various-sized bladders forming an irregular mass (resembling a grape cluster), usually without a protoscolex (Fig. 34.46). The origin of the racemose cysticercus is unknown. Some believe it may be an aberrant *T. solium* cysticercus, or be formed as the scolex disintegrates, but the bladder wall continues to grow and proliferate (similar to a metastatic cancer). Neurocysticercosis coinfection is likely to be increasingly recognized in patients with HIV and should be included in the differential diagnosis of CNS infections in these patients.

Hymenolepis (*Rodentolepis*) *nana*

Infection is usually acquired by the ingestion of infective *Hymenolepis nana* eggs, primarily from human stool (direct life cycle). The eggs hatch in the stomach or small intestine, and the liberated larvae, or oncospheres, penetrate the villi in the upper small intestine. The larvae develop into the cysticercoid stage in the tissue and migrate back into the lumen of the small intestine, where they

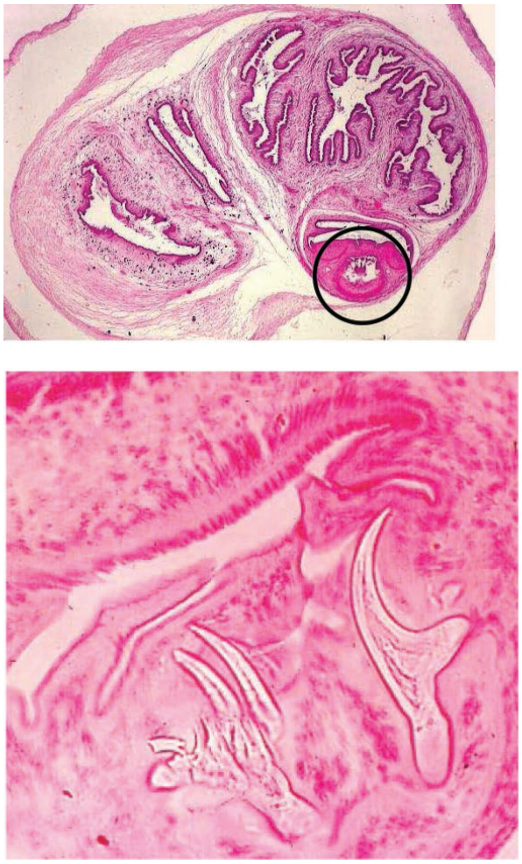

Figure 34.45 Cysticercus. (Upper) Scolex of a cysticercus showing a sucker and hooklets of *Taenia solium* (circle); note the hooklets are barely visible. (Lower) Enlarged image of the hooklets within the cysticercus. (Armed Forces Institute of Pathology photographs.) doi:10.1128/9781555819002.ch34.f45

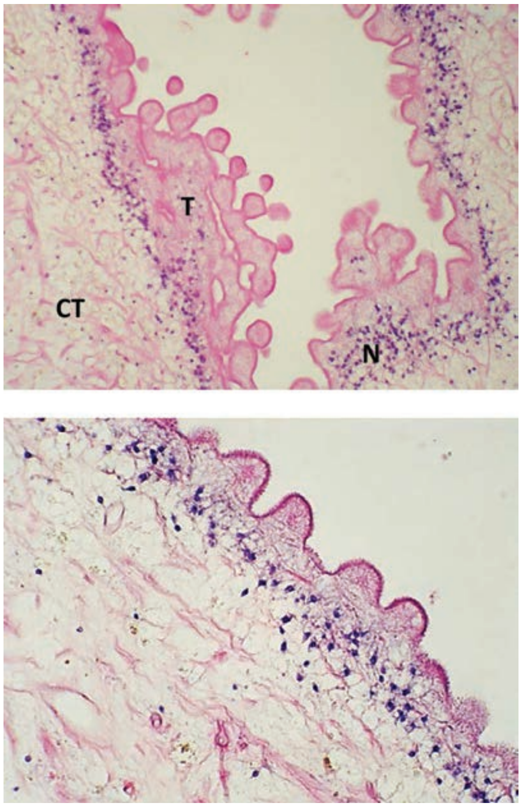

Figure 34.46 *Taenia solium* racemose cyst in the brain. (Upper) The bladder wall of the racemose cysticercus has three separate layers. The tegumental surface has wart-like protuberances and is acidophilic (T). Beneath the tegument are small, rounded, pyknotic nuclei (N). The innermost layer is made up of loose connective tissue (CT). (Lower) Higher magnification. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
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attach to the mucosa (Fig. 34.47). The adult worms mature within several weeks. They are very small compared with worms of the *Taenia* species and measure up to 40 mm long. The fact that an intermediate host is not required in the life cycle was determined in the late 1800s. For this reason, *H. nana* has been considered to be the most common tapeworm throughout the world, and it is estimated that 50 million to 75 million people worldwide are infected. The infection is most commonly seen in children, although adults are also infected.

Echinococcus granulosus Hydatid Disease (Cystic Disease)

The most common site for hydatid disease in humans is the liver (60 to 70% of cases) (Fig. 34.48 and 34.49). Although not all oncospheres survive passage through the body, those that do survive begin to grow and develop. Usually by the fifth month, the wall of the hydatid cyst has become differentiated into an outer friable, laminated, nonnucleated layer and an inner nucleated germinal layer. Various daughter cysts (brood capsules) bud off from the inner germinal layer and may remain attached or float free in the interior of the fluid-filled cyst. The individual protoscolices bud off from the inner wall of the daughter cysts; these protoscolices and free daughter cysts are called hydatid sand. Each scolex normally invaginates to protect the hooklets. Although not every cyst produces daughter cysts and/or protoscolices, this general tissue organization is called a unilocular cyst, in which the cyst contents are held within a single limiting cyst wall.

The metacestode of *Echinococcus granulosus* is a white, spherical, fluid-filled cyst that can vary from a few millimeters to many centimeters in size, containing brood capsules with protoscolices. The cyst wall has multiple layers. The thin inner layer (germinal membrane) possesses

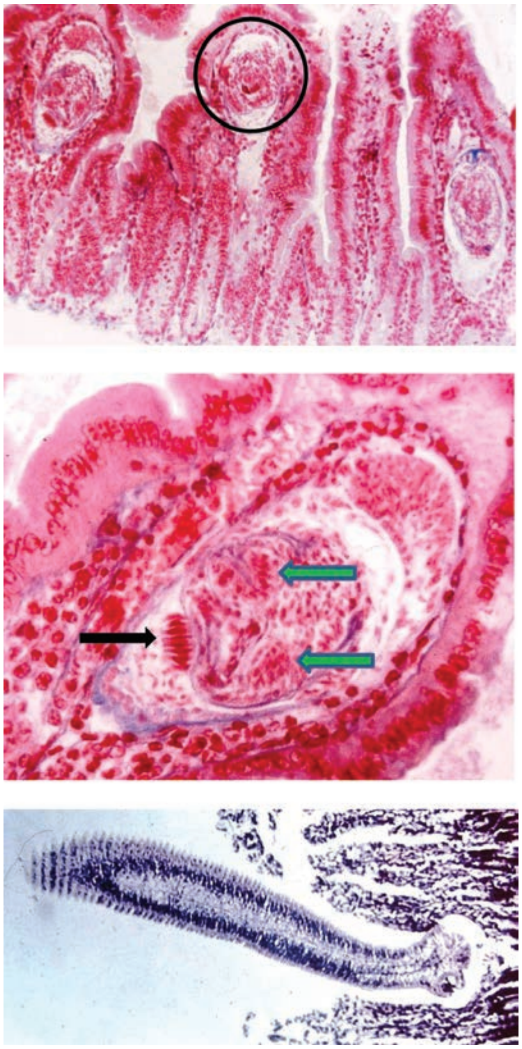


Figure 34.47 *Hymenolepis nana* cysticerci in the villi of a mouse. (Top) When mature, these cysticerci (circle) emerge, attach to the intestinal wall, and develop into the adult worm (same life cycle seen in humans). (Middle) Note the curved suckers (green arrows) and hooklets (black arrow). (Bottom) Section through *H. nana* embedded in the mucosa. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of B. Gueft; specimen courtesy of M. Yoeli.) doi:10.1128/9781555819002.ch34.f47

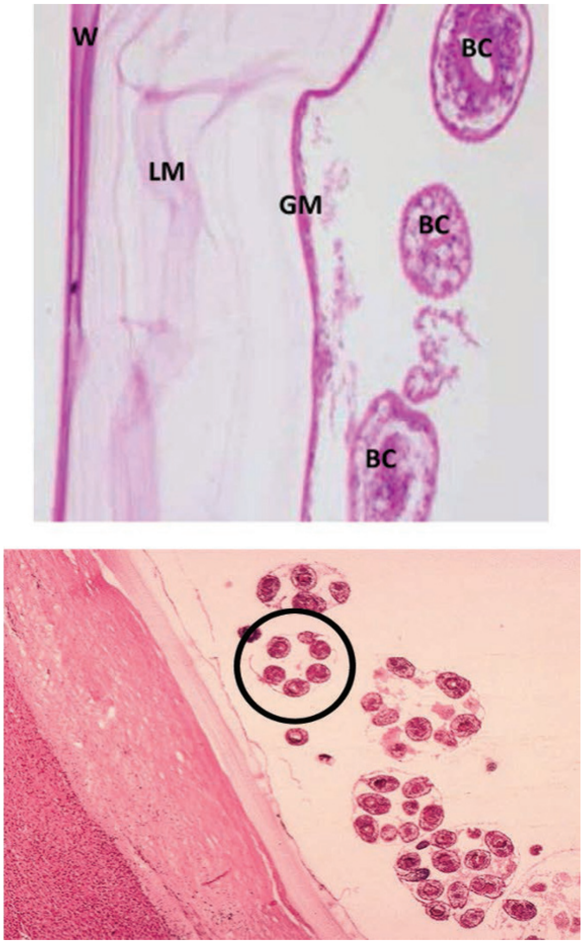


Figure 34.48 (Upper) *Echinococcus granulosus* brood capsules (BC) arising from the germinal membrane (GM). The fibrinous wall of the host (W) and the laminated membrane (LM) are also seen. (Armed Forces Institute of Pathology photograph.) (Lower) Hydatid cyst; note the layers and brood capsules filled with protoscolices (circle) (courtesy of CDC Public Health Image Library, Peter Schantz). doi:10.1128/9781555819002.ch34.f48

nuclei and occasionally calcareous corpuscles. The outer layer (laminated membrane) is approximately 1 mm thick. Although the layering can be seen with H&E stain, it is more distinct using Gomori methenamine-silver stain (GMS). The protoscolices are ovoid and measure approximately 100 μm; each one contains four suckers, many calcareous corpuscles, and two rows of hooklets, one large and one small. The hooklets can be stained (GMS, acid-fast, Ryan blue modified trichrome), but appear birefringent. Most are invaginated; however, some may be evaginated. Some cysts in humans are sterile and do not produce brood capsules, daughter cysts, or protoscolices.

Severe symptoms can occur if the cyst ruptures spontaneously, from trauma, or during surgery. In addition to the spread of tissue from which additional hydatid cysts can grow, there may be serious allergic reactions, including rash, anaphylactic shock, or death. Cyst rupture can also lead to cholangitis and cholestasis. Morbidity and mortality rates associated with perforated hydatid cysts are higher than those associated with nonperforated cysts. Lung, brain, spinal, and orbital hydatid cysts have been more commonly seen in younger patients. It is thought that age somehow alters the host-parasite relationship and thus af-

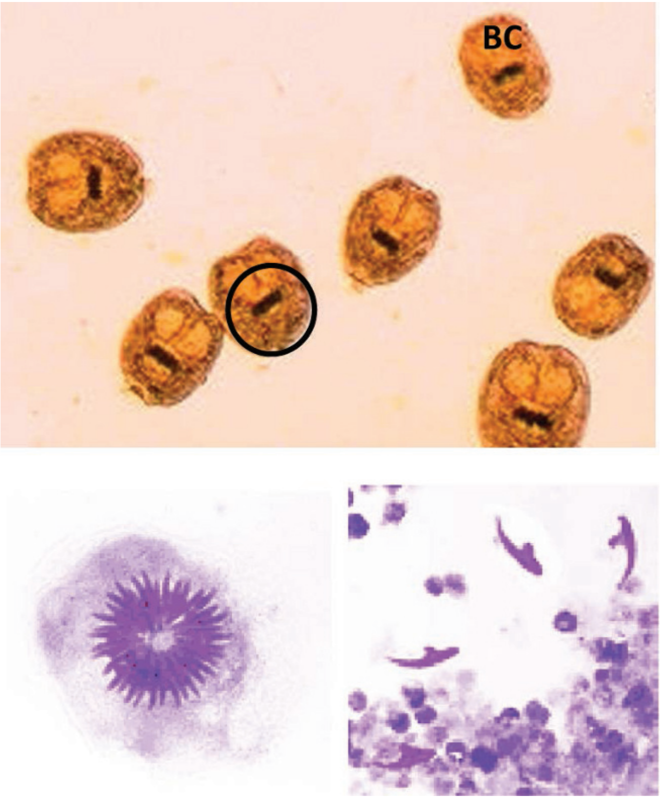


Figure 34.49 *Echinococcus granulosus*. (Upper) Brood capsules (BC); the dark areas represent the hooklets on the protoscolices (circle). (Lower) Hooklets stained with the Ryan modified trichrome/blue. (Courtesy of Norbert Ryan.) doi:10.1128/9781555819002.ch34.f49

fects the organ distribution of the cysts. Multiorgan involvement seems to be on the rise in children.

Hydatid cysts should be considered in patients with abdominal masses with no clearly defined diagnosis. Although eosinophilia is present in 20 to 25% of patients, it is merely suggestive. Many asymptomatic cysts are first discovered after radiologic studies. The cyst usually has a well-defined margin with occasional fluid level markings. These studies can also be helpful in diagnosing osseous involvement. Scans may also demonstrate a space-occupying lesion, particularly in the liver. If the cyst is large and located in the abdomen, a thrill is sometimes detected.

Echinococcus multilocularis Hydatid Disease (Alveolar Disease)

Although the alveolar form of hydatid disease has been found in other tissues (eye, bone, lung, peritoneum, adrenals, brain, ovary, lymph nodes, prostate, gallbladder, pancreas, spleen), the liver is the most common site. The disease may resemble a slowly growing carcinoma, may cause symptoms of intrahepatic portal hypertension, and may spread to other sites via metastatic dissemination. In humans there are several stages of the infection; they have been described as initial, progressive, advanced, stable, and abortive.

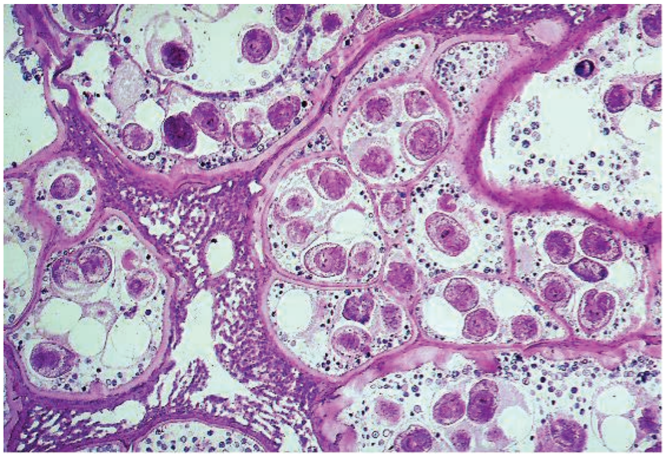
Proliferation of the cyst is most active at the advancing margin, while the interior of the cyst structure may deteriorate and appear to be a necrotic abscess filled with pus. Since the human is not a normal host, the typical brood capsules, protoscolices, and calcareous corpuscles may not develop (Fig. 34.50). In humans, the laminated membrane may surround vesicles that are void of protoscolices and other internal structures. The lesions caused by *E. multilocularis* may appear as firm, solid, cancer-like masses, and the outer layer has a poorly defined margin that continues to grow and spread throughout the tissue. In tissue sections, homogeneous, thin cystic structures of various dimensions are strongly PAS positive. Mucoid material within the cystic structures is also PAS positive. Wide coagulative necrosis is seen, as well as foreign-body-type giant cells appear at the periphery of the lesion in some cases. Necrotic ground, mucoid globules, and PAS-positive hyaline cuticular structures are evident in cytologic smears; some smears also demonstrate foreign body-type giant cells.

In a case where the patient presented with a multicystic mass in the pancreas, fine-needle aspirate biopsy revealed some protoscolices, free hooklets, and fragments of the laminated layer, findings which are pathognomonic for echinococcosis.

Taenia (*Multiceps*) spp. (*Taenia multiceps*, *Taenia serialis*) (Coenurosis)

Although the adult tapeworms resemble *Taenia* spp., the larval form is a coenurus, a unilocular cyst with the same

Figure 34.50 *Echinococcus multilocularis*. Numerous vesicles in tissue; H&E stain. Note there is no single limiting membrane, as occurs in *E. granulosus* hydatid cysts. This growth tends to mimic a metastatic cancer. doi:10.1128/9781555819002.ch34.f50



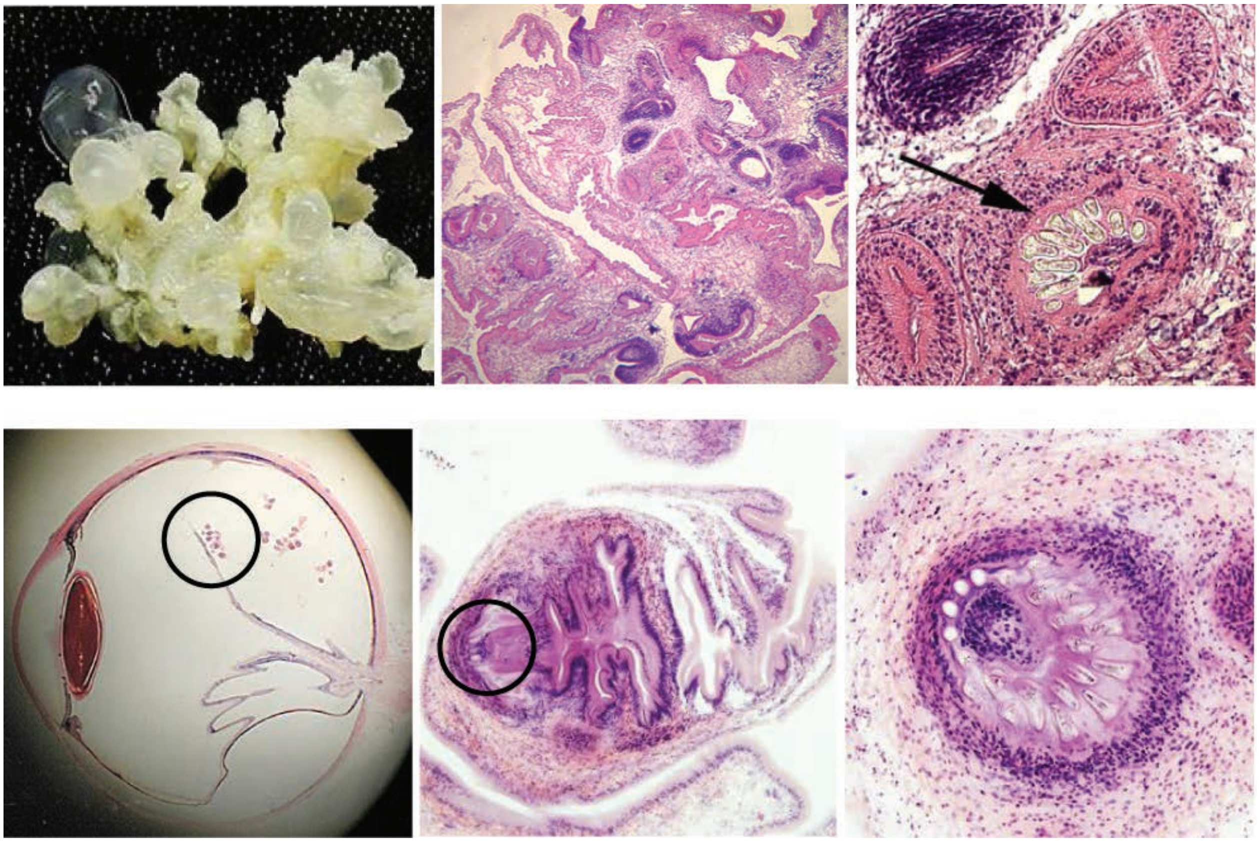


Figure 34.51 (Upper row) Coenurus (*Taenia multiceps*, *T. serialis*) removed from a subcutaneous nodule in the shoulder. (Left) Gross specimen. (Middle) Cross section. Although the species was not identified in this case, the pathology is consistent with *T. serialis*. (Right) Higher magnification (200×) of the same specimen shown on the left. The black arrow points to hooklets in one of the protoscolices. (Lower row) Coenurus from a human eye. (Left) *Taenia* species with multiple protoscolices (circle); no daughter cysts develop in a coenurus. (Middle) Higher magnification of one of the protoscolices; note the hooklets (circle). (Right) High magnification of the protoscolex showing multiple hooklets. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch34.f51

configuration as a cysticercus but containing multiple scolices. Human cases have been reported worldwide; however, identification to the species level is often very difficult if not impossible (Fig. 34.51). In humans, fewer than 175 cases have been reported. Most coenuri have been located in the brain or spinal cord, but they can also be found in the subcutaneous tissues. Symptoms have been those associated with any space-occupying lesion, including headache, vomiting, paraplegia, hemiplegia, aphasia, seizures, and eye symptoms (if the coenurus is present in the eye).

Metacestodes (coenuri) are usually thin-walled, white or gray, spherical or ovoid polycephalic cysts, measuring from a few millimeters to 6 cm. A coenurus contains gray or white jelly-like fluid and multiple yellow-white protoscolices. The numerous protoscolices distinguish a coenurus from a cysticercus, which contains a single protoscolex. Coenuri may be multilocular, but most are unilocular.

The body wall varies from 50 to 150 μm and is composed of a tegument, muscle cells, and tegument cells. On the surface of the tegument are fine, hair-like structures called microtriches or microvilli, 1 to 2 μm long. The microvilli form a border that completely covers the outer surface of the cyst wall and the invaginated surface of each protoscolex (4).

Spirometra mansonoides and *Diphyllobothrium* spp. (Sparganosis)

The larval forms (spargana) of *Spirometra mansonoides* and *Diphyllobothrium* spp. were first recognized in humans from tissues removed at autopsy. In areas where infections are endemic, similar forms can be found in the subcutaneous connective tissue and between the muscles in frogs, lizards, snakes, birds, and certain mammals. When these spargana are fed to the definitive hosts (dogs and cats), adult tapeworms of several related species of *Spi-*

rometra develop. The general name for these spargana is *Spirometra mansonoides*. Although cases have been reported worldwide, sparganosis is most common in China, Japan, and Southeast Asia.

Human infection can occur from (i) ingestion of infected *Cyclops* spp. (the procercoid stage penetrates the intestinal wall, migrates into tissues, and develops into the sparganum), (ii) ingestion of raw infected flesh of one of the second intermediate hosts (fish, snakes, amphibians, and mammals), or (iii) local application of raw, infected flesh of one of the second intermediate hosts to the skin, conjunctiva, or vagina (the spargana migrate from vertebrate host tissues into human tissue). The spargana are white, ribbonlike worms measuring from a few millimeters to several centimeters long. Human infections with spargana are classified into two forms, nonproliferative sparganosis and proliferative sparganosis (aberrant spargana).

The sparganum is a wrinkled, white, ribbon-shaped organism, 3 mm in width and up to 30 cm long. It is a solid-bodied larva that has no bladder and has bothrids (two longitudinal grooves) at the anterior end instead of suckers (similar to the scolex of *Diphyllobothrium latum*). The larva has an unsegmented strobila 20 to 30 cm in length, bundles of longitudinal muscle fibers scattered throughout the mesenchyme, and a thick tegument (Fig. 34.52).

Most patients have slowly growing, tender, subcutaneous nodules. These nodules may also be migratory. Ocular sparganosis is accompanied by pruritus, pain, lacrimation, and edematous swelling of the eyelid (which may look much like Romaña's sign in Chagas' disease). Depending on the location of the sparganum, there may be elephantiasis (lymph channels), peritonitis (intestinal perforation), or brain abscess. Most patients have leukocytosis and eosinophilia. The sparganum has been reported to live for more than 9 years.

The more elongate, wormlike structure of the sparganum can usually be distinguished from the bladder structure of a cysticercus or coenurus, since the last two have suckers and hooklets, both of which are lacking in a sparganum. The parasites are usually recovered alive and are approximately several centimeters in length. They are glistening white and opaque, they resemble narrow tapeworm proglottids, and they are motile, similar to the motility seen with a *Taenia saginata* proglottid.

Trematodes

Schistosoma mansoni, *S. japonicum*, *S. mekongi*, *S. malayensis*, *S. haematobium*, *S. intercalatum* (Blood Flukes)

Schistosoma parasites are blood flukes that infect an estimated 200 million people worldwide (Fig. 34.53 and 34.54). In chronic infection with *Schistosoma*, the severe pathology, including liver fibrosis and splenomegaly, is

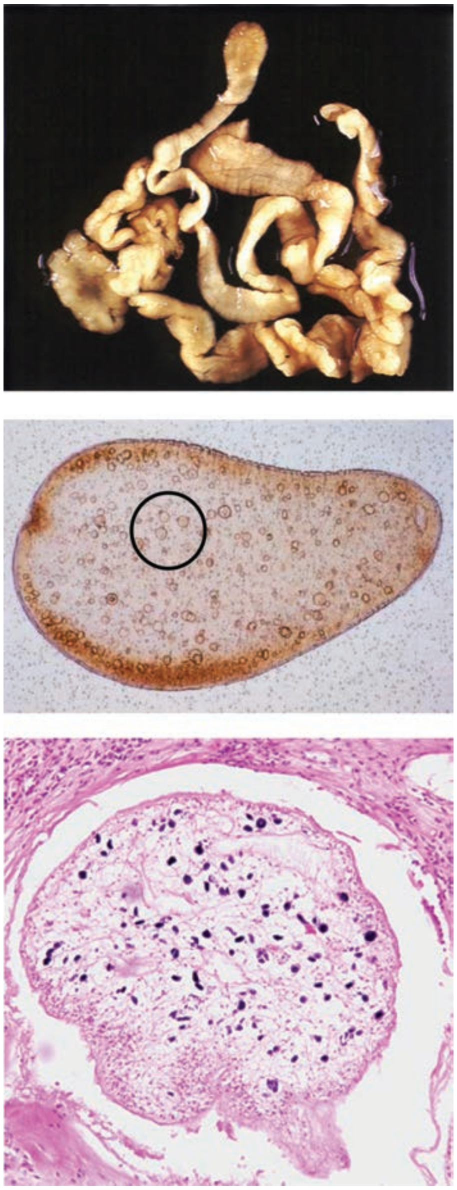


Figure 34.52 *Spirometra* sparganum larva. (Top) Spargana are larval forms of various tapeworms in the genus *Spirometra*. Spargana are white and ribbonlike in shape, range from a few millimeters to >30 cm in length, and are actively motile. The solid sparganum lacks suckers and bladder walls; there are irregular bundles of muscle fibers, typical folded tegument, and calcareous corpuscles in the parenchyma (Armed Forces Institute of Pathology photograph). (Middle) *Spirometra mansonoides* sparganum; note the calcareous corpuscles, which are refractile sand-like particles in the parenchyma (circle) (courtesy of R. E. Pugh, http://parasite.org.au/pugh-collection/Spirometra-%20mansonoides%204%2021.jpg_Index.html). (Bottom) Proliferating sparganum in groin tissue of a patient from Paraguay; H&E (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch34.f52

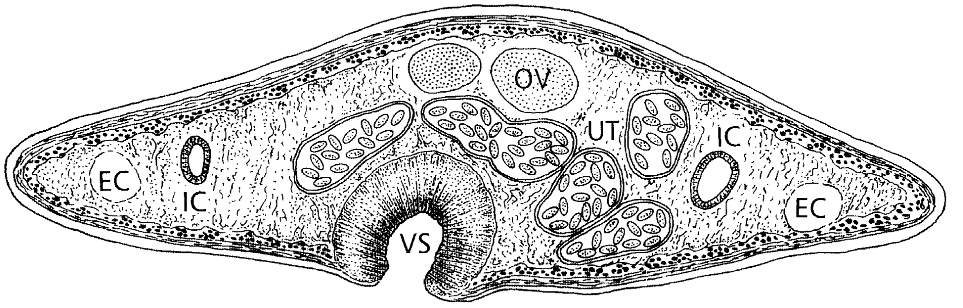


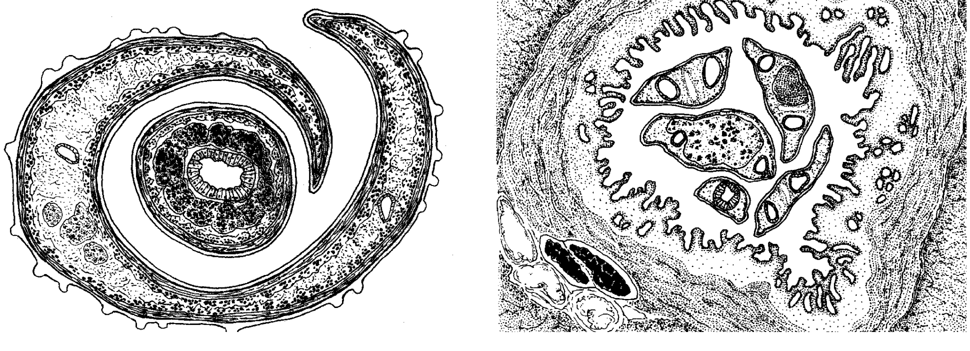
Figure 34.53 Diagram of the cross section of a trematode. Note the coiled uterus (UT) containing eggs, the lobed ovary (OV), intestinal ceca (IC), and excretory canals (EC). Also, note the large ventral sucker (VS). (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch34.f53

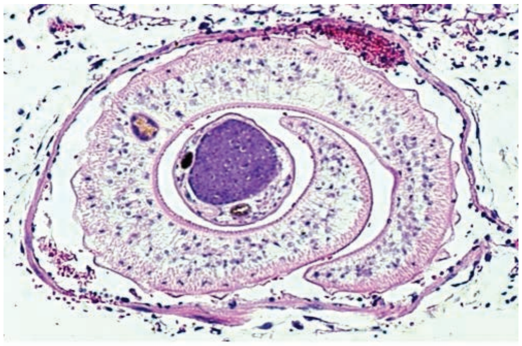
caused by the immune response to the parasite eggs rather than the parasite itself (Fig. 34.55, 34.56, and 34.57). One of the clinical manifestations of helminth infection is the presence of type 2 granulomas. The lung is the primary site of organ involvement in a range of granulomatous conditions. The immune response to *Schistosoma mansoni* eggs in mice results in the development of hepatic, intestinal, and pulmonary granulomas that lead to extensive fibrosis. The cellular composition of the granulomas includes eosinophils, alternatively activated (M2) macrophages, lymphocytes, neutrophils, mast cells, and fibroblasts. Further, the recruitment and migration of these cells into the site of inflammation are controlled by cytokines and chemokines. Granulomas vary in structure; some eggs are surrounded by an eosinophilic material, the Splendore-Hoeppli phenomenon. Immature and dead eggs provoke little to no response.

Schistosome eggs are highly immunogenic. Within infected hosts, eggs induce vigorous immune responses and are rapidly surrounded by inflammatory cells, creating a granuloma. Neutrophils are believed to play a significant role in *Schistosoma japonicum* granulomatous pathology. In the initiation of granuloma formation, neutrophils are recruited to the core of the granuloma, leading to a neutrophil-mediated inflammatory response which ultimately causes tissue damage. At a later stage, neutrophils are recruited again and accumulate at the periphery of the granuloma, where they release a number of granule proteins involved in collagen degradation and reabsorption. It is well known that granuloma formation is a T-cell-mediated immune response. The T-cell-mediated response, especially CD4$^+$ T-cell response, has been reported to participate in neutrophil induction in schistosomiasis.

Eggs of *S. haematobium* and *S. japonicum* often become calcified and surrounded by hyalinized scar tissue. While alive in the veins, adult worms provoke no host response; however, if they die, the dead worms cause large focal lesions. In some patients with *S. mansoni* infection, eggs may be numerous in the mucosa, lamina propria, and submucosa. Many eggs laid in the mesenteric venules are

Figure 34.54 (Left) Diagram of male and female *Schistosoma* trematodes. The female worm is lying with the male worm *in copulo*. Note the small tuberculations on the dorsal tegument of the male worm. (Right) Sections of adult *Clonorchis sinensis* in the common bile duct. Note that in different sections, different structures are visible, including the intestinal ceca, the ovary, and parts of the testis. Also note the marked proliferation of the bile duct epithelium. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch34.f54





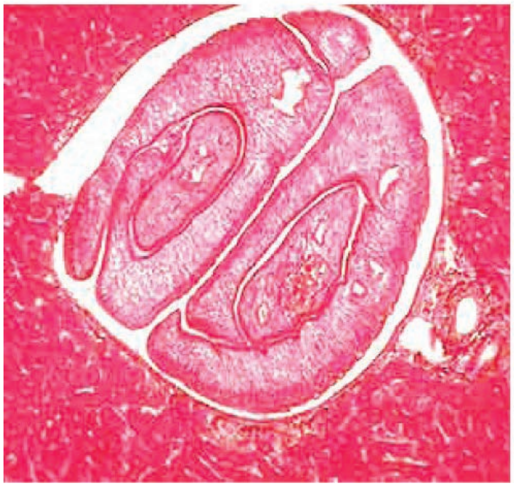


Figure 34.55 (Upper) Cross section of a *Schistosoma mansoni* adult worm pair in the mesenteric venule; note the smaller female worm is lying within the gynecophoral canal of the male worm (courtesy of the National Library of Medicine, National Institutes of Health). (Lower) *Schistosoma* adults in tissue. Note that the smaller female worm is lying within the gynecophoral canal of the male worm. The intestinal ceca are visible within both male and female worms. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
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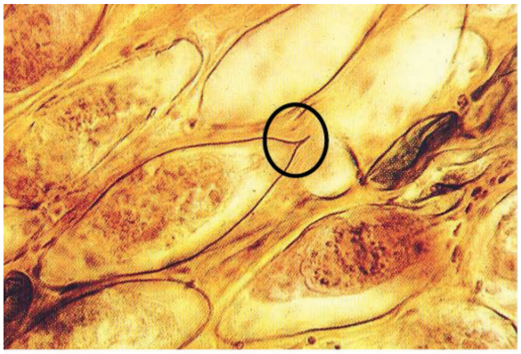

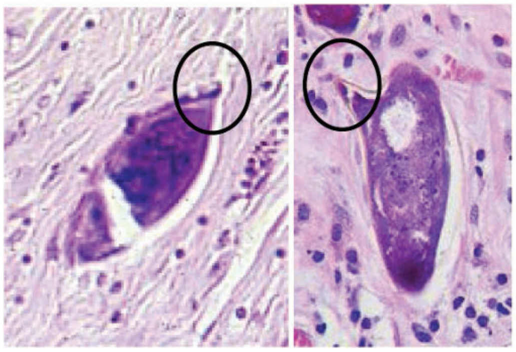

Figure 34.56 (Upper) *Schistosoma haematobium* eggs in bladder biopsy; note the large terminal spine (circle). (Lower, left) *S. haematobium* eggs in bladder biopsy, note the terminal spine (circle); (right) *S. mansoni* in liver tissue. (Courtesy of Munaf Desai, Al Qassini Hospital, Shatjah, United Arab Emirates, and the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch34.f56

carried into the intrahepatic portal circulation. The granulomas formed can result in marked portal fibrosis.

It is important to remember that if *S. haematobium* eggs are seen in urine, some may be in the process of hatching and some may have already hatched, releasing the miracidium larvae (Fig. 34.57).

Paragonimus spp. (Lung Fluke)

Paragonimus westermani is found in Asia, *Paragonimus mexicanus* is an important human pathogen in Central and South America, and *Paragonimus kellicotti* infections are found in North and South America. Most paragonimiasis infections are caused by *P. westermani*. A number of other species are thought to infect humans. Humans are infected by ingesting uncooked crabs or crayfish containing metacercariae. The metacercariae excyst in the duodenum and migrate through the intestinal wall into the abdominal cavity. The larvae migrate around or through the diaphragm into the pleural cavity and the lungs and mature to adults in the vicinity of the bronchioles, where they discharge their eggs into the bronchial secretions.

Migration of the larval forms through the intestinal wall into the abdominal cavity is generally not associated with any significant pathologic changes or symptoms. If the larvae remain in the abdomen, some patients may have abdominal pain, intra-abdominal masses, tenderness, fever, diarrhea, nausea, vomiting, and eosinophilia. Once the larvae have reached the peritoneal cavity, they begin to migrate through organs and tissues, producing localized hemorrhage and leukocytic infiltrates.

When the worms finally reach the lungs and mature, a pronounced tissue reaction occurs, with infiltration of eosinophils and neutrophils. A fibrotic capsule forms around the worm. The cysts contain purulent fluid with flecks or “iron filings” composed of brownish yellow eggs. Many of the cysts perforate into the bronchioles, releasing their contents of eggs, necrotic debris, metabolic by-products, and blood into the

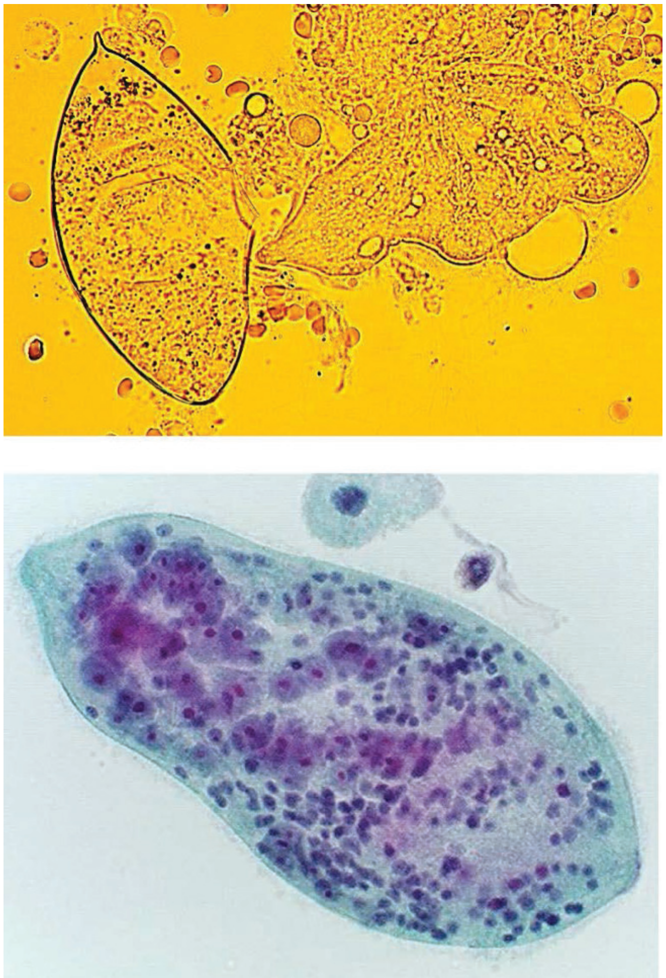

Figure 34.57 (Upper) *Schistosoma haematobium* egg in urine; note the egg shell on the left and the hatched miracidium on the right (courtesy of Aaron Hill, Virginia Commonwealth University Medical Center). (Lower) Stained miracidium larva, hatched egg. Note the anterior end in both images.
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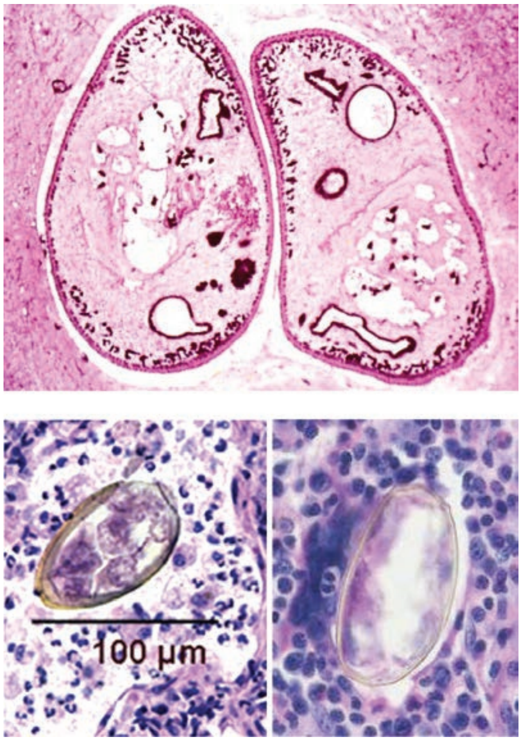

Figure 34.58 (Upper) Two *Paragonimus westermani* parasites within a lung cavity. Note the dark, small spots of the vitellaria just within the tegument. The dark openings represent the ceca, with clear portions of the uterus. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) (Lower) *Paragonimus* sp. from lung biopsy; H&E. Eggs measured 80 to 90 µm by 40 to 45 µm, but were not identified to species. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch34.f58

respiratory tract. The eggs may also enter the pulmonary tissue, or they may be carried by the circulatory system to other body sites, where they cause a granulomatous reaction (Fig. 34.58).

Symptoms of paragonimiasis depend largely on the worm burden of the host and are usually insidious in onset and mild in patients with chronic infections. Light infections may be asymptomatic, although peripheral blood eosinophilia and lung lesions may be noted on X-ray examination. As the cysts rupture, a cough develops, with increased production of viscous blood-tinged sputum (rusty sputum, which may have a foul fish odor) and increasing chest pain.

Larval forms may end up in many ectopic sites other than the lungs and have been detected in the liver, intestinal wall, muscles, peritoneum, and brain. The most serious consequences of paragonimiasis are the cerebral complications, which are commonly found in younger age groups. Unlike adult flukes in other extrapulmonary sites, worms found in the brain usually contain eggs. The worms probably migrate from ruptured lung cysts and travel through the soft tissues surrounding veins into the brain area. The worms eventually encapsulate, but before being walled off they cause necrosis within the brain tissue, as well as possible cerebral hemorrhage, edema, and meningitis.

Skull radiographs generally reveal round or oval cystic calcifications; they have been described as looking like bubbles. CT scans reveal multilocular cysts with edema, migration tracks, peripheral density, bronchial wall thickening, and centrilobular nodules; ring enhancement may be visible after the use of contrast (chapter 19, Fig. 19.19). MRI demonstrates areas of granulomatous inflammation surrounding the lesions; MRI of the brain may reveal multiple conglomerated iso- or low-signal-intensity round nodules with peripheral rim enhancement. In the liver, CT and MRI images may reveal a cluster of small cysts with rim enhancement in the subcapsular area. This finding seems to be characteristic for hepatic paragonimiasis. Solitary nodular lesions can often mimic lung cancer, tuberculosis, or fungal diseases. Three characteristic findings are seen with cerebral paragonimiasis: arachnoiditis, encapsulated abscesses, and granulomas that may calcify.

Fasciola spp. (Sheep Liver Fluke)

This infection is an important health problem, particularly in the Andean countries of Peru, Bolivia, and Chile. Humans are infected by ingestion of uncooked aquatic vegetation on which metacercariae are encysted. Metacercariae excyst in the duodenum and migrate through the intestinal wall into the peritoneal cavity. The larvae enter the liver by penetrating the capsule (Glisson's capsule) and wander through the liver parenchyma for up to 9 weeks. The larvae finally enter the bile ducts, where they mature and produce eggs, which are passed out in the feces. The adult worms can attain a length of 30 mm and a width of about 13 mm and can live for more than 10 years.

In the acute phase, symptoms may be present over a period of weeks to months and include dyspepsia, fever, right upper quadrant pain, anorexia, hepatomegaly, splenomegaly, ascites, urticaria, respiratory symptoms, and jaundice. Metacercarial larvae do not cause significant pathologic damage until they begin to migrate through the liver parenchyma (Fig. 34.59). The amount of damage depends on the worm burden of the host. Linear lesions of 1 cm or greater can be found. Hyperplasia of the bile ducts occurs, possibly as a result of toxins produced by the larvae. Symptoms associated with this migratory phase have included fever, epigastric and right upper quadrant pain, intestinal complaints, and urticaria, although other patients remain asymptomatic. Asymptomatic infections appear to be more common in Peru. Other symptoms include loss of appetite, flatulence, nausea, diarrhea, and a nonproductive cough. Patients may also have hepatomegaly, splenomegaly, ascites, chest signs, and jaundice. Leukocytosis, eosinophilia, and mild to moderate anemia are found in many patients.

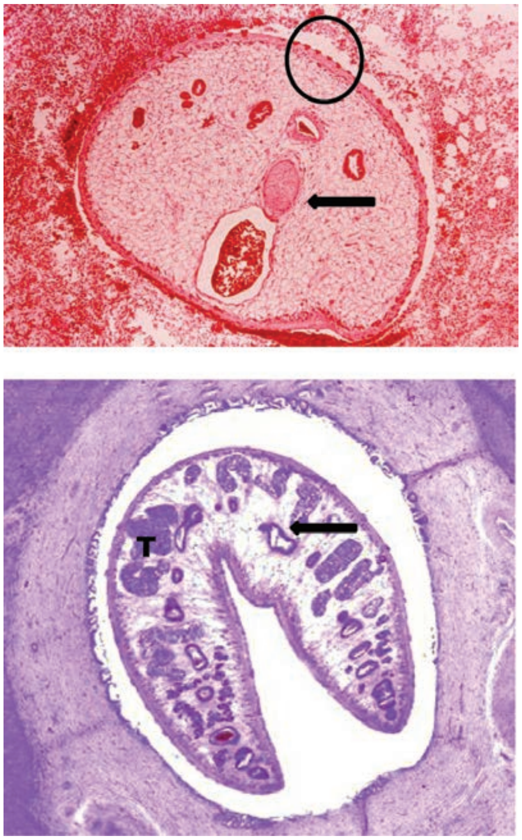

Figure 34.59 (Upper) Adult *Fasciola hepatica* embedded in the intestinal wall. The parasite presents as an oval to round mass limited by a spined cuticle (circle). The parasitic parenchyma is punctuated by multiple sections through the gut. The two centrally located tubular structures, separated by a light-staining muscular sucker (arrow), are part of the reproductive apparatus of the parasite. A marked inflammatory response is present in the adjacent host tissues, and fresh RBCs can be seen near the parasite. (Lower) Adult *F. hepatica* in the liver. Note that the intestinal ceca (arrow) and portions of the testes are clearly visible (T). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman; photograph courtesy of B. C. Walton.)
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In the chronic phases, the patient has few to no symptoms once the flukes have lodged in the biliary passages. However, there may be some epigastric and right upper quadrant pain, diarrhea, nausea, vomiting, hepatomegaly, and jaundice. If the flukes are found in the extrahepatic biliary ducts, symptoms may mimic those seen in cholelithiasis. In the chronic phase, there tend to be some liver function abnormalities, as well as eosinophilia. Larvae may be found in ectopic foci after penetrating the peritoneal cavity. Worms in human infections have been discovered in many areas of the body other than the liver. Other body sites include the intestinal wall, lungs, heart, brain, and skin. Symptoms mimic those seen with VLM and include vague abdominal pain. Unfortunately, surgery may be required to determine the exact cause of the symptoms.

Once the worms have established themselves in the bile ducts and matured, they cause considerable damage from mechanical irritation and metabolic by-products as well as obstruction. The infection produces hyperplasia of the biliary epithelium and fibrosis of the ducts with portal or total biliary obstruction. The gallbladder undergoes similar pathologic changes and may even harbor adult worms. Adult worms may reinvade the liver parenchyma, producing abscesses. Symptoms and signs of infection during the late stages after egg production has begun are those associated with biliary obstruction and cholangitis. Acute epigastric pain, fever, pruritus, jaundice, hepatomegaly, and eosinophilia are common.

Clonorchis sinensis (Chinese Liver Fluke)

Humans become infected by ingesting the metacercariae in raw or incompletely cooked fish. Metacercariae excyst in the duodenum, enter the common bile duct, and travel to the distal bile capillaries, where the worms mature over a period of about a month (Fig. 34.60). The life cycle takes approximately 3 months to complete in humans. Most patients with mild infections of fewer than 100 flukes have few symptoms, which may include general malaise, abdominal discomfort, and diarrhea. In 10 to 40% of patients, peripheral eosinophilia occurs with fluctuating obstructive jaundice. Moderate infections with fewer than 1,000 worms include symptoms of fever and chills, fatigue, anorexia, diarrhea, weight loss, discomfort, and abdominal distention. In severe disease with up to 20,000 flukes, an additional symptom may include acute right upper quadrant pain. In the late stage of severe cases, jaundice, diarrhea, portal hypertension, hepatosplenomegaly, ascites, and edema can occur.

Figure 34.60 *Clonorchis sinensis*. (Upper) Cross sections of adult flukes within the bile duct, which shows marked hyperplasia of epithelium. (Lower) Adult fluke in bile duct, larger magnification. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
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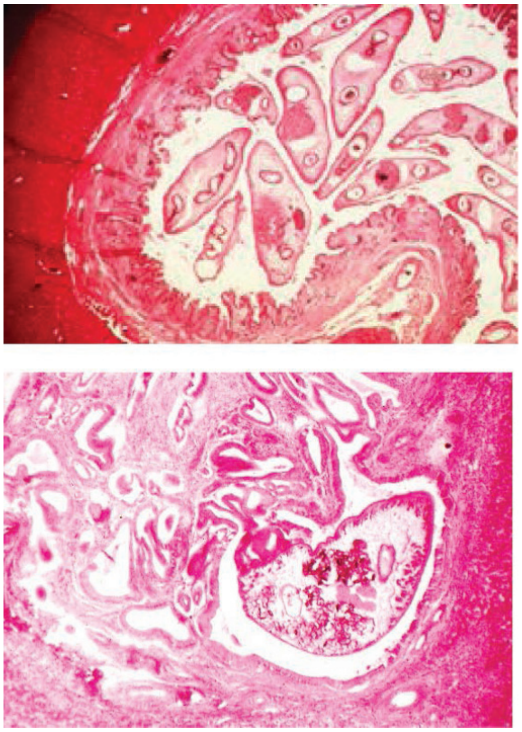


In general, complications result from biliary obstruction. As the worms mature in the distal bile ducts, an inflammatory response is seen in the biliary epithelium. The extent of pathologic changes is related to the intensity and duration of infection. The lesions are confined mainly to the biliary system and are the result of mechanical irritation and toxins produced by the worms. In light infections, there appears to be little or no change in liver parenchyma, whereas heavy infections cause thickening and localized dilations of the bile ducts with hyperplasia of the mucinous glands. As a result, the biliary tract may become obstructed, causing bile retention, infiltration of lymphocytes and eosinophils, and fibrosis. The adenomatous changes may persist for many years in patients with light infections. The infections have been associated with obstructive jaundice, which may be aggravated by biliary stones and liver abscesses. Many patients infected with *Clonorchis sinensis* have recurrent pyogenic cholangitis. There is no direct evidence that infection with *C. sinensis* causes chronic bacterial infection. Acute pancreatitis, cholecystitis, and cholelithiasis may be the result of worm invasion. Cirrhosis is probably related to malnutrition rather than parasitic infections. CT evaluation has shown that in the presence of diffuse mild intrahepatic bile duct dilation, enlargement of the body or tail (or both) of the pancreas, with a cluster of small cystic changes within the pancreatic parenchyma, provides strong evidence of *C. sinensis* pancreatitis.

C. sinensis has been linked to neoplasms of the bile duct and to cholangiocarcinoma, which is most frequently observed in areas where clonorchiasis is endemic. Cholangiocarcinoma is a malignant tumor that arises from the bile duct epithelium and is the second most prevalent liver cancer after hepatocellular carcinoma. The tumor usually occurs in patients 60 to 80 years of age and rarely in patients younger than 40 years. There appears to be no direct link between infection and carcinoma, although one of the first steps in malignant transformation may be induced by the biliary tract hyperplasia caused by the worms. Patients with primary sclerosing cholangitis (PSC) have a substantial predisposition for bile duct carcinoma. Although the exact mechanisms are not well defined, longstanding inflammation can lead to cholangiocarcinoma in patients with chronic *C. sinensis* infection. PSC is an uncommon disease, characterized by stricturing, fibrosis, and inflammation of the biliary tree, which is closely associated with chronic inflammatory bowel disease, particularly ulcerative colitis. The only effective treatment for PSC is orthotopic liver transplantation, which in the absence of cholangiocarcinoma has a 5-year survival rate of 89%. However, patients with cholangiocarcinoma who undergo liver transplantation have a high risk of recurrence and poor survival statistics.

Peripheral cholangiocarcinoma is usually seen as a large, well-defined hepatic mass with lobulated margins. CT and MRI reveal band-like peripheral contrast enhancement of variable thickness around the tumor during the early phase, with progressive filling of contrast material in the later stages. This appearance occurs through slow diffusion of contrast materials into the interstitial spaces of the tumor. Fibrosis within this type of cholangiocarcinoma also contributes to the delayed contrast enhancement of the tumor. Additional findings in peripheral cholangiocarcinoma include capsular retraction and dilatation of the peripheral intrahepatic ducts.

Most hilar and extrahepatic cholangiocarcinomas cause a focal stricture of the bile duct, whereas papillary carcinoma is occasionally found in the form of a polypoid mass within the duct. By thin-section CT, this tumor type can be seen as an ill-defined focal wall thickening. Using ultrasonography, the tumor can appear as a thickening or encircling mass along the bile duct wall. In a number of cases, the infiltrating tumor is seen by either ultrasonography or CT as a simple nonunion of the dilated right and left hepatic ducts without any identifiable mass. Cholangiography shows a focal or diffuse stricture or complete obstruction of the bile ducts; from this image the extent of the disease can be determined.

Radiological findings are regarded as pathognomonic for clonorchiasis because they demonstrate the unique pathological changes of this disease. Due to remarkable advances in imaging technology, individual flukes can now be seen by means of noninvasive radiological techniques.

Echinostoma spp.

A number of species of echinostomes have been reported to infect humans, including *Echinostoma hortense*, *E. ilocanum*, *E. macrorchis*, *E. perforiatum*, and *E. revolutum*. Most of the species are found in Asian countries; *E. ilocanum* is the most important species. These flukes are distinguishable from other trematodes by the distinctive horseshoe-shaped collar of spines on the lateral and dorsal sides of their oral sucker (Fig. 34.61) (4).

Adult worms are attached to the mucosal wall of the small intestine and lay eggs, which are passed from the intestinal lumen in the feces. The eggs are immature, ellipsoidal, yellow-brown, and operculated. They measure 86 to 116 μm by 58 to 69 μm. The miracidia take 1 to 2 weeks to mature in the environment before they hatch from the eggs and infect the snail intermediate host. Two or more generations of rediae are produced before cercariae develop. These cercariae encyst in freshwater mollusks, and humans are infected by eating these mollusks raw rather than cooked. The metacercariae hatch in the intestine, attach to the mucosal wall, and develop into mature adult worms.

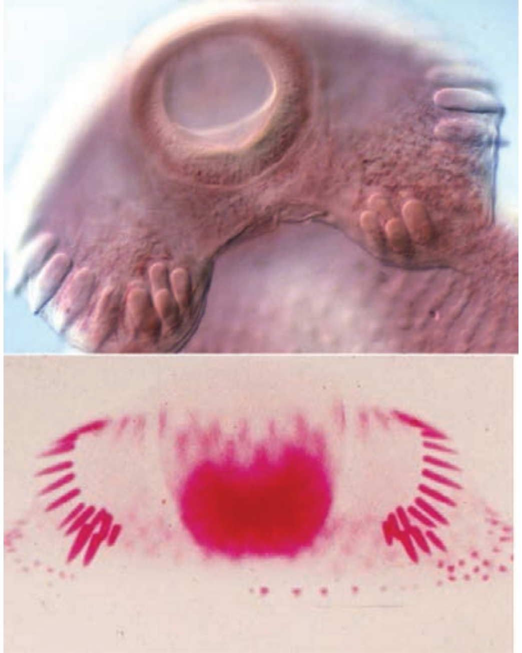


Figure 34.61 *Echinostoma* adult worm. (Upper) Stained adult worm characterized by having a circumoral disk with a crown of spines surrounding the small oral sucker (courtesy of www.k-state.edu). (Lower) Enlarged image of the crown of spines (surrounding the oral sucker). The living worm is reddish gray and measures 2.5 to 6.5 mm in length by 1 to 1.35 mm in width. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch34.f61

With light infections, the patient may be asymptomatic and the adult worms cause only minor problems other than localized inflammation. In heavy infections, the worms can produce catarrhal inflammation and mild ulceration and the patient may experience diarrhea and abdominal pain.

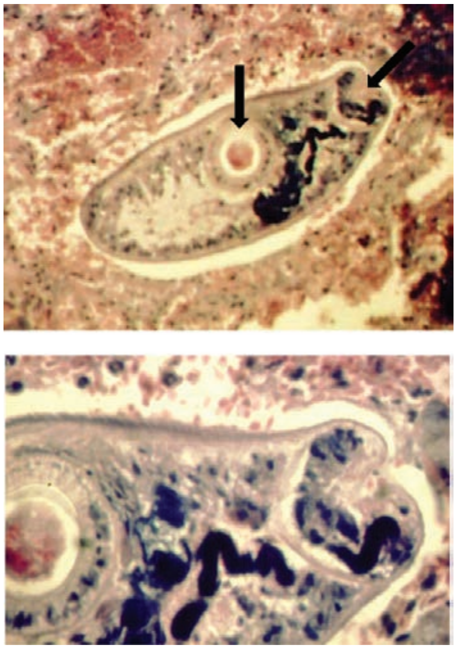
Eggs of *E. ilocanum* can be detected in the stool. They are the same size (some strains) and shape as the eggs of *Fasciola hepatica* and *Fasciolopsis buski*. Because of the overlap in size range, exact identification cannot be made from the eggs; therefore, the diagnosis may have to be made on the basis of patient history or clinical findings.

Alaria americana

Alaria americana is a trematode that is normally found in the intestines of wild mammalian carnivores such as wolves, foxes, raccoons, bobcats, and skunks. The eggs are passed in the stool, hatch in water, and invade freshwater snails of the genus *Helisoma*. Cercariae then leave the snail and infect frog tadpoles. The first case of disseminated human infection by the mesocercarial stage of this worm was found in a 24-year-old man in Canada who died 8 days after the onset of his illness. The infection was probably acquired through ingestion of inadequately cooked frogs, which serve as intermediate hosts for this parasite. The infection was diagnosed by lung biopsy, and mesocercariae were found in the stomach wall, lymph nodes, liver, myocardium, pancreas and surrounding adipose tissue, spleen, kidney, lungs, brain, and spinal cord at autopsy (Fig. 34.62). Apparently, there was no host reaction to the parasites. Granulomas were present in the stomach wall, lymph nodes, and liver, but no worms were recovered from these sites.

Two cases of eye infection with mesocercariae of *Alaria* were found in unrelated Asian men who had unilateral decreased vision. Both patients had pigmentary tracks in the retina, areas of active and/or healed retinitis, and other signs of diffuse unilateral subacute neuroretinitis. The worms were seen in the retinas and vitreous several years after the original infection was thought to have occurred. The probable source of infection was ingestion of undercooked frogs' legs containing the infective stage. In addition to the prolonged intraocular infection, the mesocercariae caused diffuse unilateral subacute neuroretinitis, a condition thought to be caused only by intraocular nematode larvae.

Figure 34.62 *Alaria americana* mesocercaria in an autopsied human liver. (Upper) Note the evidence of both the oral sucker and the acetabulum (ventral sucker) (arrow). (Lower) *A. americana*. Note the oral and ventral suckers (higher magnification). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman. Photograph courtesy of R. S. Freeman.) doi:10.1128/9781555819002.ch34.f62



Blood Parasites

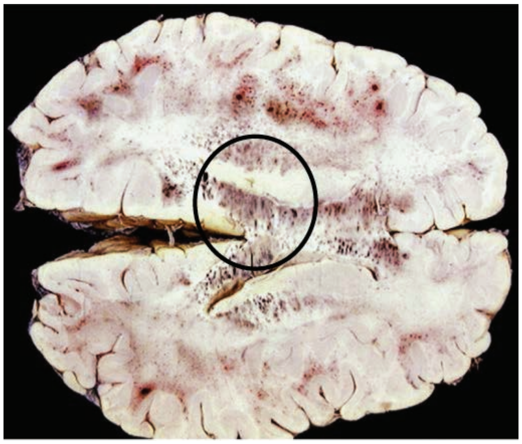
Malaria

Plasmodium spp. (Cerebral Malaria)

With the exception of tuberculosis, malaria kills more people than any other communicable disease in the world. Severe or fatal complications of *P. falciparum* malaria can occur at any time during the infection and are related to the plugging of vessels in the internal organs, with the symptoms depending on the organ(s) involved. The severity of the complications in a malaria infection may not correlate with the parasitemia seen in the peripheral blood, particularly in *P. falciparum* infections.

Cerebral malaria is most often seen in *P. falciparum* malaria, although it can occur in the other types as well (Fig. 34.63 and 34.64). If the onset is gradual, the patient may become disoriented or violent or may develop severe headaches and pass into coma. Some patients, even those who exhibit no prior symptoms, may suddenly become comatose. Physical signs of CNS involvement are quite variable, and there is no real correlation between the severity of the symptoms and the peripheral blood parasitemia. It has been shown that patients with cerebral malaria were infected with RBC rosette-forming *P. falciparum* and that plasma from these patients generally had no antirosetting activity. A rosette usually consists of a parasitized RBC surrounded by three or more uninfected RBCs. Interaction with adjacent uninfected RBCs in rosettes appears to be

Figure 34.63 Malaria. Section through the cerebrum showing more congestion of the white matter (petechial hemorrhages) than of the gray (circle). (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch34.f63



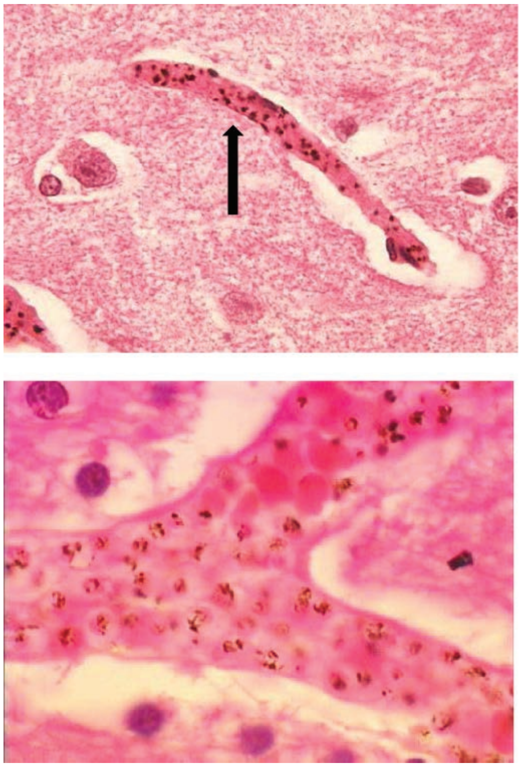


Figure 34.64 (Upper) Cerebral malaria showing cerebral capillaries congested with parasitized erythrocytes (arrow). Parasitized erythrocytes stick to the endothelium and occlude the capillaries. (Lower) Note the capillary containing parasitized erythrocytes; note the brownish malarial pigment (higher magnification). (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch34.f64

mediated by knobs seen on the parasitized RBC. In contrast, *P. falciparum* parasites from patients with mild malaria lacked the rosetting phenotype or had a much lower rosetting rate. Also, antirosetting activity has been detected in the plasma of these patients. These findings strongly support the idea that RBC rosetting contributes to the pathogenesis of cerebral malaria while antirosetting antibodies offer protection against these clinical sequelae.

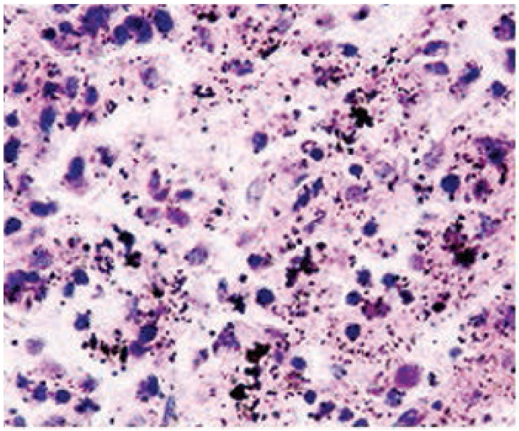


Figure 34.65 Malarial pigment present in the spleen. The spleen is enlarged to about 500 g during an acute attack; it is soft, diffusely pigmented, and congested and has rounded edges. The phagocytic cells lining the splenic sinuses contain malarial pigment, free parasites, and parasitized erythrocytes. During an acute attack, malarial pigment is evenly distributed throughout the spleen. doi:10.1128/9781555819002.ch34.f65

Signs and symptoms of cerebral malaria include headache, nuchal rigidity, altered levels of consciousness, seizures, ataxia, aphasia, and dysarthria. Because *P. falciparum* is the only malarial parasite that becomes sequestered in vessels, it is the only species of *Plasmodium* that has been seen in tissue sections. Malarial pigment is the end product of hemoglobin digestion into a porphyrin conjugated with a protein derived from the globin portion of hemoglobin. Malarial pigment and formalin pigment are morphologically very similar. Both appear microscopically as brown or black crystals that are birefringent under polarized light. However, they can usually be distinguished by certain general characteristics: malarial pigment appears as intracellular round granules. Postmortem findings of falciparum malaria usually include prominent generalized passive congestion. Grossly, there is often gray or brown discoloration of the brain, liver, and spleen caused by malarial pigment (Fig. 34.65).

Microscopically, masses of erythrocytes fill the lumen of small and medium-sized blood vessels, distending the vessels. Parasitized erythrocytes tend to lie against the endothelial surface of vessels. In a ring hemorrhage, a small vessel is occluded by parasitized erythrocytes, surrounded by necrotic parenchyma, and surrounded by hemorrhage of nonparasitized erythrocytes. In falciparum malaria, renal corticomedullary capillaries may contain parasitized erythrocytes. Pigment grains may be seen within glomeruli or phagocytic cells. In patients with oliguria, tubular necrosis and hemoglobin casts may be prominent.

Leishmania

Once leishmaniae have been carried to the bone marrow, spleen, and liver, a granulomatous cell-mediated immune response occurs that can result in subclinical disease and self-cure or in the clinical syndrome of visceral leishmaniasis. The incubation period usually ranges from 2 to 6 months; however, it can take a few days or weeks to several years. When giving a history, many patients do not remember having a primary skin lesion. In areas of endemic infection, the onset may be gradual, with vague symptoms and a general feeling of ill health. The onset in naive patients

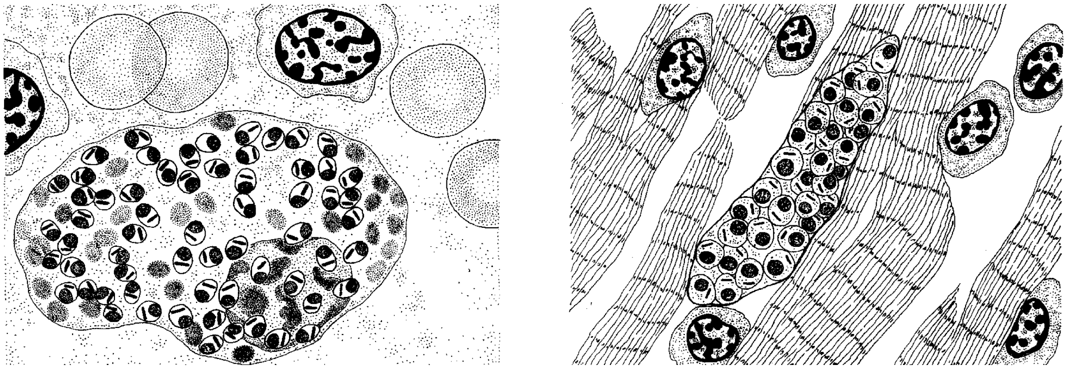


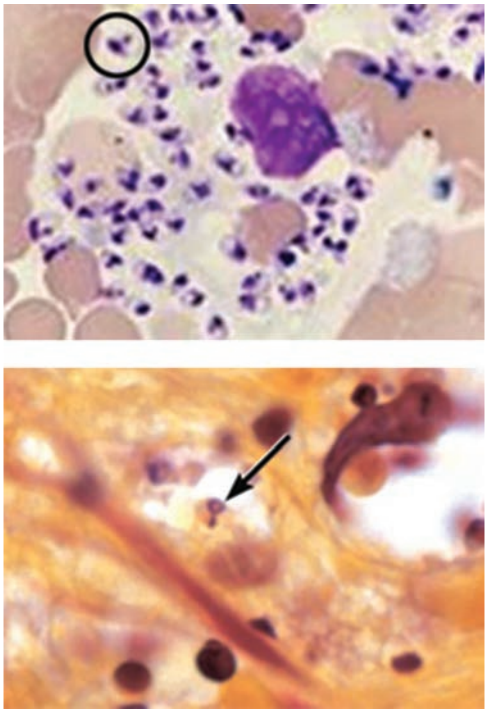
Figure 34.66 Diagram of amastigotes. (Left) *Leishmania donovani* amastigotes in a spleen smear. Each amastigote contains the dark, round nucleus and the bar-shaped kinetoplast. When cells are packed with amastigotes, often the complete morphology for each amastigote is not visible. (Right) *Trypanosoma cruzi* amastigotes in cardiac muscle. Each amastigote contains the nucleus (round, dark) and the kinetoplast (small bar). (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch34.f66

(migrants, soldiers, or travelers to areas of endemic infection) may be acute, with high fever, chills, anorexia, malaise, weight loss, and frequently diarrhea. This syndrome can easily be confused with typhoid fever, malaria, or other febrile illnesses caused by bacteria or viruses. Death may occur after a few weeks or several years. Generally, untreated visceral leishmaniasis leads to death; secondary bacterial and viral infections are also common in these patients.

The diagnosis of leishmaniasis can be difficult or sometimes impossible to confirm with standard Giemsa-stained smears. Because factors such as specimen quality, staining methods, and the skill of the microscopist can dramatically affect the sensitivity of this diagnostic procedure, a negative smear does not necessarily exclude a diagnosis of leishmaniasis (Fig. 34.66 to 34.68). In histologic sections, amastigotes stain well with H&E or Brown-Hopps modified tissue Gram stain. The Brown-Hopps stain creates the least amount of background staining and the strongest contrast from surrounding tissue, which makes identifying amastigotes much easier (1). Other stains sometimes used to identify amastigotes in tissue, including Giemsa, Wilder's reticulum, PAS, and immunoperoxidase stains, offer no advantage over the Brown-Hopps stain. Amastigotes in visceral leishmaniasis appear slightly smaller and less distinct than those observed in cutaneous leishmaniasis. Because kinetoplasts are much more difficult to identify, special stains such as Brown-Hopps and Wilder's reticulum are recommended.

Microscopically, liver sinusoids are dilated and show prominent amastigote-laden Kupffer's cells and little or no cellular reaction. Splenic aspiration is the surest method of parasitologic diagnosis, but carries the risk of splenic laceration. Careful observation of the patient after the procedure is essential. Giemsa-stained splenic smears demonstrate amastigotes in 98 to 100% of patients, and quantitation of splenic smears is helpful in measuring response to

Figure 34.67 *Leishmania* spp. (Upper) Smear of bone marrow showing many intracellular and extracellular *Leishmania* organisms. The amastigotes (Leishman-Donovan bodies) (circle) appear larger in smears than in tissue sections. The nucleus (longer dark object within the circle) and kinetoplast (smaller dark object within circle) are distinct in these organisms. (Lower) Single amastigote in the dermis (arrow) stained with Brown-Hopps tissue Gram stain. Note spherical nucleus and rod-shaped kinetoplast (Armed Forces Institute of Pathology photograph). doi:10.1128/9781555819002.ch34.f67



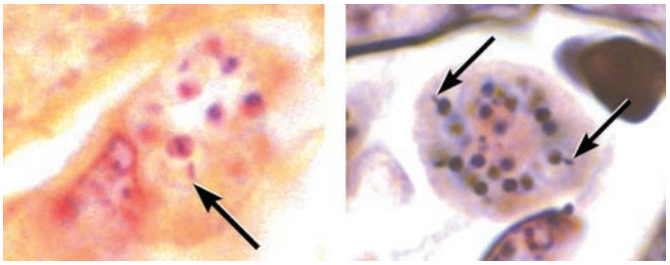


Figure 34.68 *Leishmania donovani*. (Left) Section of liver showing dilated sinusoids and greatly enlarged Kupffer cells (arrow); Brown-Hopps tissue Gram stain. (Right) Numerous black amastigotes in Kuppfer's cells; note kinetoplasts (arrows); Wilder's reticulum stain. (Armed Forces Institute of Pathology photographs.) doi:10.1128/9781555819002.ch34.f68

therapy. Promastigotes can be seen within 2 to 7 days in Schneider's medium or 7 to 21 days in Novy-MacNeal-Nicolle (NNN) medium after incubation at 25°C. For clinicians unfamiliar with splenic aspiration or concerned about its risks, there are satisfactory alternatives. Bone marrow smears usually contain amastigotes, though fewer than in splenic smears; parasites are found in up to 85% of patients.

Trypanosomes

Trypanosoma cruzi (American Trypanosomiasis, Chagas' Disease)

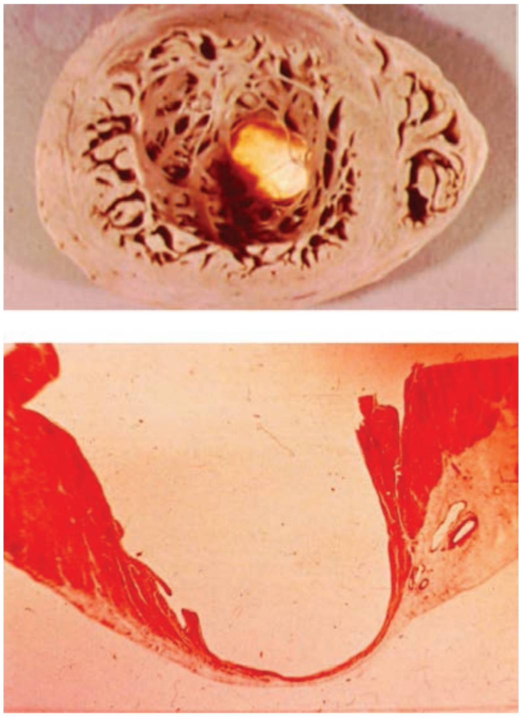
The disease is transmitted to humans through the bite wound caused by reduviid bugs (triatomids, kissing bugs, or conenose bugs). Humans are infected when metacyclic trypomastigotes are released with the feces while the insect is taking a blood meal and the feces are rubbed or scratched into the bite wound or onto mucosal surfaces such as eyes or mouth, an action stimulated by the allergic reaction to the insect's saliva. The organisms can also be transmitted as congenital infections, by blood transfusion, or by organ transplantation. On entry into the body, the metacyclic forms invade local tissues, transform to the amastigote stage, and begin to multiply within the cells. The local inflammatory process continues, forming the primary lesion, the chagoma, which blocks the lymphatic capillaries and causes edema.

On invasion of a cell, the trypomastigote loses its flagellum and undulating membrane and divides by binary fission to form an amastigote. The amastigote continues to divide and eventually fills and destroys the infected cell. Both amastigote and trypomastigote forms are released from the cell. The amastigote is indistinguishable from those found in leishmanial infections. It is 2 to 6 μm in diameter and contains a large nucleus and rod-shaped kinetoplast that stains red or violet with Giemsa stain. The cytoplasm stains blue. Only the trypomastigotes are found free in the peripheral blood.

Chronic Chagas' disease may develop years or decades after undetected infection or after the diagnosis of acute disease. The most frequent clinical sign of chronic Chagas' disease in 25 to 30% of patients is cardiomyopathy manifested by cardiomegaly and conduction changes. This may present as congestive heart failure, thromboembolic phenomena, cardiac arrhythmias, and sudden death. Cardiac pathology includes mononuclear inflammatory infiltrate, focal myocarditis, epicarditis, and neuroganglionitis, associated with variable focal fibrosis and widely variable autonomic dysfunction. Apparently, the immune inflammatory response causes the autonomic dysfunction, which may lead to life-threatening arrhythmias and sudden death (Fig. 34.69 and 34.70).

Microscopically, there is marked interstitial fibrosis of the myocardium and rare amastigotes, with focal infiltrates of lymphocytes, monocytes, and plasma cells in which multinucleated giant cells are occasionally present. Focal accumulations of neutrophils and an occasional eosinophil may also be found. Apical lesions regularly appear as segments of acellular fibrous tissue associated with epicardial vessels and adipose tissue (13).

Figure 34.69 *Trypanosoma cruzi*. (Upper) Transillumination of apex highlights the apical aneurysm. (Lower) The thinned ventricular wall shows muscle fiber loss and fibrosis most prominent near the aneurysm. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f69



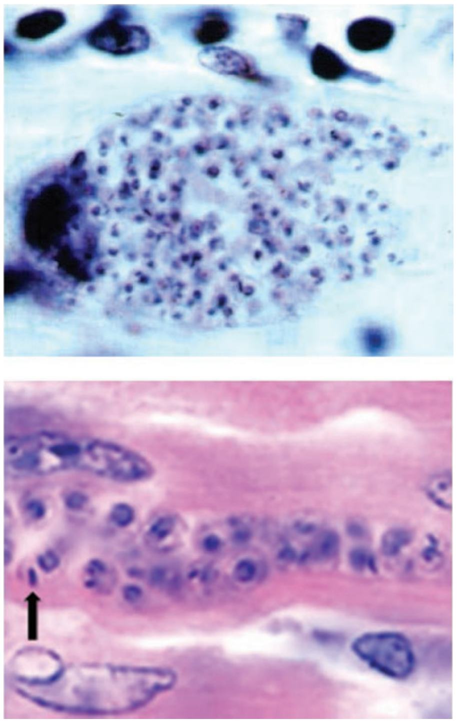


Figure 34.70 *Trypanosoma cruzi*. (Upper) Amastigotes in cardiac tissue. Note that many organisms are present; however, it is difficult to see differentiation between the nuclei and kinetoplasts. (Lower) Higher magnification of the amastigotes in cardiac muscle; note one of the kinetoplasts (looks like a small bar) (arrow). doi:10.1128/9781555819002.ch34.f70

Filaria

Wuchereria bancrofti

Infective larvae enter the skin of the human through the mosquito puncture wound when a blood meal is taken. The infective larvae enter the peripheral lymphatic system and migrate to lymph vessels distal to the lymph nodes, where they grow to mature female and male adults and mate. Thousands of developing embryos can be found within the uteri of the female. Microfilariae are released from the gravid female and can be detected in the peripheral circulation in 8 to 12 months postinfection; however, filariasis without microfilaremia is not uncommon.

Adult worms are minute and threadlike, have a smooth cuticle, and are found in the lymph nodes and lymphatic channels. Adult males are about 40 mm long by 0.1 mm in diameter. Adult females are 80 to 100 mm long by 0.24 to 0.30 mm in diameter. To perpetuate their life cycle, sheathed microfilariae invade the blood; sometimes they can also be found in hydrocele fluid and chylous urine. The microfilariae range from 244 to 296 µm long and actively move about in the lymph or blood. The microfilaria has a sheath, and the body nuclei do not extend to the tip of the tail.

Early symptoms of filariasis include high fevers (filarial or elephantoid fever), lymphangitis, and lymphadenitis. Filarial fever usually begins with a high fever and chills that last 1 to 5 days before spontaneously subsiding, and in many cases, patients with filarial fevers do not have a microfilaremia. The lymphangitis extends in a distal direction from the affected nodes where the filarial worms reside.

Lymphadenitis and lymphangitis develop in the lower extremities more commonly than in the upper. However, in some areas of endemic infection such as northeastern Brazil, factors other than filarial worms can be the cause of subclinical pathology of the leg lymphatics and are not specific for bancroftian filariasis. In addition to limbs, there can be genital (almost exclusively a feature of *W. bancrofti* infection) and breast involvement. The lymph nodes most often affected are the epitrochlears and femorals. The nodes are firm, discrete, and tender and tend to remain enlarged, while the lymph vessel is indurated and inflamed. The overlying skin is tense, erythematous, and hot, and the surrounding area is edematous. Occasionally, abscesses form at the lymph node or along the lymphatic system and may take 2 to 3 months to heal.

In lymphatic vessels that harbor the worms, an inflammatory reaction includes the infiltration of plasma cells, eosinophils, and macrophages in and around the affected vessel. Repeated inflammatory attacks lead to hyperplasia of the endothelium in addition to cellular infiltration. There is an increase in hydrostatic pressure resulting from the damage to the lymph vessel, which causes an increase in vessel wall permeability. The chronic leakage of fluid containing high levels of protein into the surrounding tissues (lymphedema) results in the hard or brawny edema, with thickening and verrucous changes in the skin, also known as elephantiasis (Fig. 34.71). Histologic examination of the skin shows hyperkeratosis and acanthosis, with scarring and a loss of dermal elasticity. Regional lymph nodes proximal to the worms eventually become fibrotic, and the proximal lymph vessels become stenotic and obstructed, leading to the development of collateral lymph channels. Elephantoid tissue is a mixture of lymph and fat in a matrix of fibrotic tissue.

Onchocerca volvulus (River Blindness)

Onchocerca volvulus L3 larvae are transmitted by black flies and develop into adults that are found in subcutaneous nodules. Adult female and male worms lie within fibrous tissue capsules in the dermis and subcutaneous tissues. The female adult is very long and thin, measuring up to 50 cm by 0.3 mm, while the adult male is 2.5 to 5 cm by 125 to 200 µm. Gravid females produce from 1,000 to 3,000 microfilariae per day, which invade the subcutaneous tissues, skin, and eyes.

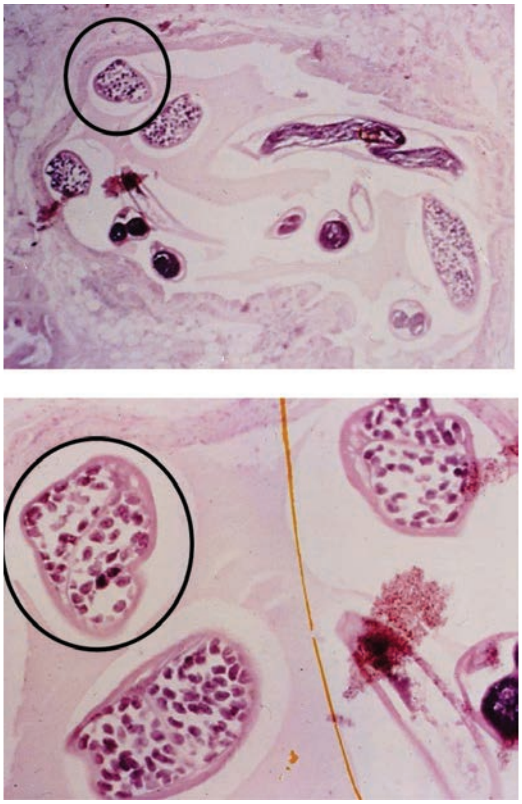


Figure 34.71 *Wuchereria bancrofti.* (Upper) Multiple sections of the worm in a dilated lymphatic vessel. Note the thin, threadlike structures *in utero* (circle). Various lengths are seen, depending on the actual tissue cut. Although these helminths are nematodes, they are shown with the blood parasites, since blood is the primary specimen for diagnostic purposes. (Lower) At higher power, the developing microfilariae *in utero* (circle) and cuticle are seen. (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f71

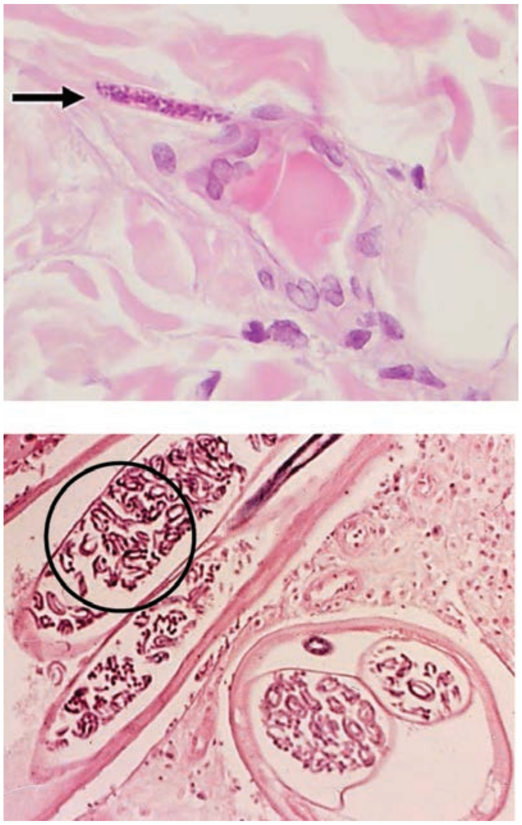


Figure 34.72 *Onchocerca* sp. (Upper) Microfilaria of *Onchocerca* sp. (arrow) detected in biopsied skin in the interstitium of the dermis; H&E stain. (Lower) The gut is seen as a cross section in the lower portion of the image and longitudinally in the upper portion; note the curvy microfilariae in different lengths, depending on the direction of the cut (circle). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f72

The prepatent period in humans is 3 to 15 months. Light infections may produce no symptoms, and worms may be free in the tissue; however, in heavy infections, major disease manifestations include dermatitis, onchocercomas (subcutaneous nodules containing adult worms), lymphadenitis, and blindness. Even though there is an inflammatory reaction that causes the formation of a fibrotic capsule (onchocercoma) around the adult, the main pathology appears to be directed against the microfilariae (Fig. 34.72).

Acute attacks of onchodermatitis have resulted in a purplish skin discoloration on the trunk and upper limbs known as mal morado in Central America. Chronically infected skin loses its elasticity and becomes hypertrophic or thickened. As a result of atrophy and loss of skin elasticity, patients may develop premature exaggerated wrinkling of the skin. Skin may become hypo- or hyperpigmented. In Africa, patients may exhibit localized areas of spotty depigmentation surrounded by slightly hyperpigmented zones, occurring mainly on the shins; this condition is known as leopard skin.

A major complication of onchocerciasis has been ocular lesions leading to bilateral blindness. The prevalence of blindness depends on the organism strain and the prevalence of disease within an area, while the degree of eye involvement depends on the duration and severity of infection. Visual problems can include fluffy corneal opacities, sclerosing keratitis, and iridocyclitis leading to glaucoma and cataracts; chorioretinitis and optic atrophy also occur. Eye damage is caused by living and dead microfilariae in the cornea, anterior and posterior chambers, iris, retrolental space, vitreous, choroid, retina, sclera, and optic nerve. Progressive pathology generally develops over time, with resulting blindness seen in adults.

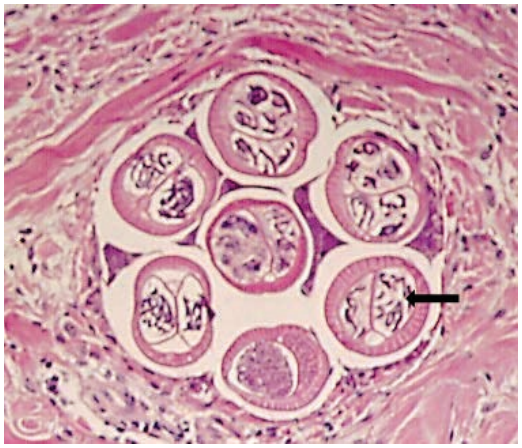


Figure 34.73 *Mansonella streptocerca* in a section of skin. Note the long, slender microfilariae *in utero* (arrow). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.) doi:10.1128/9781555819002.ch34.f73

Mansonella streptocerca

Until recently, *Mansonella streptocerca* was named *Dipetalonema streptocerca*. Adult female and male worms live in the dermal tissues, and the microfilariae are found in the skin, where they can be ingested by *Culicoides* spp. (midges), the insect vectors. Pruritic dermatitis with hypopigmented macules on the skin and lymphadenopathy have been common complaints.

Microfilariae can be found primarily in the upper third of the dermis in the dermal collagen (Fig. 34.73). Changes in the skin include sclerosis of dermal papillae, edema, incontinence of melanin, fibrosis, and cellular infiltrates around blood vessels, and the lymphatics are frequently dilated. In untreated patients, adult worms are occasionally found in the dermis; they do not tend to cause an immune response when alive.

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Appendix 34.1 Histologic identification of parasites[a]

Body site	Organisms	Stain	Comments
Intestine	Protozoa		
	Entamoeba histolytica (cecum, ascending colon, sigmoid rectal region; other sites less common); trophozoites and cysts are normally passed in stool	H&E, PAS, trichrome or iron hematoxylin in stool	Differential between organism and histiocyte: in an organism, the nucleus may not be visible; in histiocytes, a portion of the nucleus is almost always visible; both trophozoites and histiocytes stain red with PAS (review nuclear details); histolysis of invaded tissue; flask-shaped ulcers; organisms seen in healthy ulcer margins, not in central, necrotic areas
	Giardia lamblia (duodenum); trophozoites and cysts are normally passed in stool; organisms can be seen in duodenal aspirates or by using other sampling methods (Entero-Test)	H&E, Giemsa, trichrome or iron hematoxylin in stool	Normal to mild to severe blunting of villi; loss of the brush border; infiltration of lamina propria with lymphocytes and granulocytes; chronic inflammation; tissue invasion does not occur; trophozoites may appear as sagittal sections of small, crescentic organisms that may adhere to the epithelial surface; front views will reveal the typical teardrop shape
	Balantidium coli; trophozoites and cysts are normally passed in stool; tissue invasion may occur in the colon and appendix	H&E, trichrome or iron hematoxylin in stool (may be difficult to see on permanent stained smears; wet mount is best)	Simple hyperemia to marked ulcerations (large intestine); very large size (trophozoites); presence of cilia, large cytostome, and prominent macronucleus will distinguish these ciliates from other protozoa; due to large size, cut planes may not reveal all internal morphology; early lesion resembles that of *E. histolytica*, but the opening into the mucosa is larger
	Cryptosporidium spp. have been recovered from mouth to anus, more common in small intestine; oocysts (4–6 µm) are passed in stool; disseminated disease can occur, with oocysts being passed in sputum	H&E, modified acid-fast stains for stool (oocysts positive)	Mild to moderate villous atrophy, increased crypt size, mild to moderate mononuclear cell infiltrates of lamina propria; organisms along brush border are small and measure 3–5 µm; other coccidia and the microsporidia develop within the cells and so are not confused with the brush border location of *Cryptosporidium* spp.
	Cyclospora cayetanensis developmental stages have been seen in jejunal enterocytes, usually at the luminal end rather than at the brush border; tends to resemble developmental stages of *Cystoisospora belli*	H&E, modified acid-fast stains for stool (oocysts positive, but staining variable)	Acute and chronic inflammation, disruption of surface epithelium, villous atrophy, and crypt hyperplasia are seen; intraepithelial organisms within parasitophorous vacuoles, primarily in apical areas; electron microscopy reveals enterocyte vacuolization and many intraepithelial reactive cells.
	Cystoisospora belli (more common in small intestine); oocysts are passed in stool; unsporulated oocysts measure 20–33 µm long by 10–19 µm wide and may contain a single sporoblast (usually seen in cases of diarrhea)	H&E, modified acid-fast stains for stool (oocysts positive)	Same as *Cryptosporidium* spp., but development occurs within the epithelial cells; tissue eosinophilia can be seen

(continued)

Appendix 34.1 Histologic identification of parasites[a] *(continued)*

Body site	Organisms	Stain	Comments
	Microsporidia; identification of organisms to the genus and species levels requires electron microscopy, tissue culture, immunofluorescence studies, or PCR; differentiation of spores from bacteria or small yeasts in stool is extremely difficult	H&E, tissue Gram stains, PAS, silver stains; modified trichrome and calcofluor white used for stool or urine	Developing spores seen within enterocytes; sizes of individual spores are ~1–3 μm in humans; *Enterocytozoon bieneusi* and *Encephalitozoon* (*Septata*) *intestinalis* involved, primarily seen in the compromised host; both organisms can disseminate to other organs and spores can be found in liver, lungs, kidneys, CNS, and cornea
	Trypanosoma cruzi (esophagus, colon); trypomastigote may be found in peripheral blood in the early stage of infection	H&E	Disease of ganglion cells of mesenteric plexuses; can cause severe distention and thinning of visceral wall; scarring, chronic inflammation; amastigotes may shrink, nuclei and kinetoplasts may not be visible in tissue sections and may resemble *Histoplasma*; tissues most often involved include brain, cardiac, and smooth muscle cells
	Nematodes		
	Enterobius vermicularis (cecum, appendix, other sites; may also be found in female reproductive tract or peritoneal cavity); eggs and adult worms recovered on Scotch tape preparations or pinworm paddles; stool not recommended	H&E	Characteristic lateral alae on worm cross sections; eggs *in utero* may also be visible; most often seen in appendix with little or no inflammation
	Ascaris lumbricoides (small intestine, bile ducts, liver, peritoneal cavity); fertilized and unfertilized eggs passed in stool; adult worms may also be seen in stool; migrating larvae may be seen in sputum	H&E	Large size, characteristic muscular wall formed by long, irregular muscle fibers; sectioned (eggs may be seen with their thick, bumpy shells); the body wall has a thick, multilayered cuticle, fibrous-appearing hypodermis (expands to form large lateral chords, within which are seen excretory canals); short, muscular esophagus and simple intestinal tube with an irregular lumen; larval forms may be more difficult to identify
	Strongyloides stercoralis (usually duodenum and upper jejunum; may be in any tissue in disseminated disease); rhabditiform (noninfectious) larvae usually seen in stool; filariform (infectious) larvae, as well as adult worms and eggs, may be seen in stool in hyperinfection cases	H&E	Sections may reveal adult females, well-segmented eggs, and numerous larvae; although the morphology of the adult female may be difficult to see in tissues, the small size of the worm and the presence of developing eggs and larvae close to the adult worm strongly suggest this diagnosis; with hyperinfection, the bowel lining becomes congested and edematous, with possible ulcerations; chronic infection may reveal bowel wall fibrosis
	Hookworms (*Necator*, *Ancylostoma*) (usually upper part of small intestine); hookworm eggs normally passed in stool; eggs of both genera look the same	H&E	Relatively thick cuticle and thin hypodermis; lateral hypodermal chords are usually visible; wide-open mouth sucking the mucosa; adult *Necator* has cutting plates, while *Ancylostoma* has teeth

	Trichuris trichiura (large intestine); whipworm eggs normally passed in stool	H&E	Most sections reveal both the thicker body area ("whip handle"), which lies free in the lumen, and the slender anterior area ("whip"), which is firmly embedded into the mucosa; sectioned eggs may be visible
	Trichinella spp. (duodenum, small intestine)	H&E	Edema, chronic inflammation, hyperemia, serosa petechiae, dilatation of bowel loops; prominent Peyer's patches; swollen intestinal villi, mucin secretion; diffuse infiltration of eosinophils (lamina propria); larvae may be seen and may resemble microfilariae
	Cestodes		
	Taenia saginata (most common), *T. solium*, *Diphyllobothrium latum*; proglottids and/or eggs are normally passed in the stool	H&E	May see loose proglottids and/or eggs in appendix
	Hymenolepis nana; eggs are normally passed in the stool	H&E	Both larval and adult forms may be in intestinal sections in the human
	Echinococcus granulosus (mesentery)	H&E, acid fast	External laminated, anuclear cyst wall; hooklets are acid fast
	Trematodes		
	Schistosoma mansoni, *S. japonicum*, *S. haematobium*, less common schistosomes (*S. mekongi*, *S. intercalatum*); eggs may be seen in urine and/or stool	H&E	Adults and eggs may be seen in the mesentery or mesenteric veins; egg identification may depend on demonstration of spine in section; important to determine if egg contains miracidium larva (indicates active infection); in the wall of the intestinal tract, polyp formation may occur (more eggs found in polyps)
Liver	Protozoa		
	Leishmania donovani	H&E, PAS	Organisms are dot-like in routine section; they are larger (2–4 μm) in tissue impression smear; kinetoplast next to nucleus is very characteristic and differentiates these organisms from *Toxoplasma* (no kinetoplast); *Histoplasma* is PAS positive, while *L. donovani* is PAS negative
	Toxoplasma gondii	H&E, PAS	Resting forms (bradyzoites) are pyriform with no kinetoplast (if many organisms are seen per cyst, they may appear as dots); bradyzoites within cysts are PAS positive; tissue cysts have a very thin wall; tissue cysts of *Toxoplasma* must be differentiated from *Sarcocystis* (seen most frequently in skeletal or cardiac muscle and larger than *Toxoplasma*); when reviewing animal tissues (from dogs), *Neospora caninum* may be present and will mimic *Toxoplasma*

(continued)

Appendix 34.1 Histologic identification of parasites[a] *(continued)*

Body site	Organisms	Stain	Comments
	Microsporidia	See intestine	See information on intestine
	Plasmodium falciparum, *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi*	H&E	Exoerythrocytic stages (mature tissue schizonts) measure 45–80 µm and contain 15,000–40,000 merozoites per cell; malarial pigment may be seen in Kupffer cells; capillaries may be filled with parasitized RBCs (pigment is also visible here); the host does not respond to the presence of these organisms; pigment within blood vessels can be seen with routine tissue stains, but the use of polarized light may also be helpful in demonstrating the birefringent granules of malarial pigment
	Nematodes		
	Capillaria hepatica; eggs in human stool would be from the ingestion of parasitized livers, probably from squirrels or other animals (spurious infections—eggs disappear from the feces in a few days)	H&E, trichrome (Masson)	Liver parenchyma; eggs remain in liver and the life cycle does not continue in humans; eggs resemble those of *T. trichiura*, but the shell appears beaded or pitted; polar plugs are less pronounced than in *T. trichiura*; intense granulomatous reaction around eggs; the general egg shape, the bipolar plugs, and striations in the thick shell make this identification relatively easy
	Toxocara cati or *T. canis*, *Baylisascaris* spp. (visceral larva migrans); adult worms play no role in human disease (very rare exceptions)	H&E	Liver contains largest number of larvae; any organ can be affected; eosinophilic granuloma; focal necrosis may occur; larval morphology includes single lateral ala, nonpatent intestine, and large paired excretory columns; *Baylisascaris* larvae are larger than *Toxocara* larvae and have a patent gut and a predilection for the CNS
	Cestodes		
	Echinococcus granulosus; adult worms and/or eggs found only in the small intestine of canids (primarily dogs)	H&E, acid fast, GMS, PAS	External laminated, anuclear cyst wall; thin nucleated inner layer; hooklets are acid fast; calcified cysts often reveal no hooklets; mature cysts contain brood capsules and protoscolices; degeneration of brood capsules results in "hydatid sand" (degenerating protoscolices and hooklets in the cyst fluid); some cysts are sterile
	Echinococcus multilocularis; adult worms and/or eggs found only in the small intestine of canids (primarily dogs and foxes)	H&E, acid fast, GMS, PAS	Alveolar structure with no limiting membrane; scolices seldom present, thus no protoscolices, hooklets, or calcareous corpuscles; laminated and germinal layers are collapsed, folded, and scattered throughout the liver
	Trematodes		
	Schistosoma mansoni, *S. japonicum*, *S. haematobium*, less common schistosomes (*S. mekongi*, *S. intercalatum*); eggs normally passed in urine and/or stool	H&E, trichrome (Masson)	Granulomatous tissue reaction around eggs (lymphocytes, plasma cells, eosinophils); trichrome demonstrates fibrotic tissue; eggs of *S. japonicum* more numerous in liver (greater egg-laying capacity); adult worms seen most frequently in cross or transverse sections within blood vessels in the wall of the intestine or in liver; often the female is seen *in copula*, surrounded by the larger male body

	Fasciola hepatica (bile duct); eggs normally passed in stool	H&E	Large size plus cross section of worm containing intestinal diverticula; eggs may also be seen but are usually collapsed or distorted
	Clonorchis (*Opisthorchis*) *sinensis* (bile duct); eggs normally passed in stool	H&E	May be eosinophilic infiltration and slight thickening of the duct wall; may progress to fibrosis, cirrhosis of liver, biliary obstruction, etc.; long, coiled uterus full of eggs may be seen; adult trematodes in bile ducts may suggest *C. sinensis*, *Opisthorchis* spp., *Dicrocoelium dendriticum*, and *Fasciola* spp.
Spleen	Protozoa		
	Leishmania donovani	H&E, PAS	See information on liver
	Plasmodium falciparum, *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi*	H&E	See information on liver
Gallbladder	This organ is seldom invaded by parasites; occasionally, *Cryptosporidium*, microsporidia, *Clonorchis* (*Opisthorchis*) *sinensis*, *Ascaris lumbricoides*, and *Fasciola hepatica* are found; schistosome eggs may also be found		Eggs and/or sections of adult trematodes may be seen; developmental stages of *Cryptosporidium* possible
Kidney	Protozoa		
	Microsporidia	See intestine	See information on intestine
Urinary bladder	Trematode		
	Schistosoma haematobium	H&E, acid fast	Seen within blood vessels, often near the bladder; early inflammation may progress to thickening, hyperplasia, fibrosis, and ulceration; carcinoma may be a complication; terminal egg spine may be visible; important to determine if egg contains miracidium; eggs may be distorted and collapsed; calcified eggs may also be seen; eggs are not acid fast, like those of *S. mansoni* and *S. japonicum*; adult worms may be seen *in copula* within the blood vessels
Lungs	Protozoa		
	Entamoeba histolytica	H&E	Usually extension of liver abscess in the right lower lobe; may occur elsewhere; nucleus is not always visible; should be area of histolysis around organisms
	Toxoplasma gondii	H&E	Cysts look like those in the liver; tachyzoites are crescent shaped, especially when released from the cells
	Cryptosporidium spp.	H&E	Organism morphology similar to that in intestine; organisms may be seen in sputum in disseminated disease

(continued)

Appendix 34.1 Histologic identification of parasites^{a} *(continued)*

Body site	Organisms	Stain	Comments
	Microsporidia	Tissue Gram stains, PAS, silver stains, H&E	Organism morphology similar to that in intestine; spores may be found in sputum
	Nematodes		
	Strongyloides stercoralis, *Ascaris lumbricoides*, hookworm, *Toxocara* spp., *Wuchereria bancrofti*, *Brugia malayi*, *Dirofilaria immitis*	H&E	Migrating larvae (*Strongyloides*, *Ascaris*, hookworm, *Toxocara* spp.) may be seen; microfilariae (*Wuchereria*) may cause an eosinophilic granuloma (pneumonitis, alveolar hemorrhage, edema)
	Trematodes		
	Paragonimus westermani, other *Paragonimus* spp.; eggs can be found in sputum and/or stool	H&E	Reaction by lung tissue isolates the worm in a thick, fibrous capsule containing hemorrhagic, purulent exudate and eggs; adults typically paired, surrounded by fibrous capsule; spines on tegument and typical eggs make the diagnosis relatively easy
Heart	Protozoa		
	Entamoeba histolytica	H&E, PAS	May be present; pericardial tamponade may occur
	Trypanosoma cruzi	H&E	Amastigotes are morphologically identical to those of *L. donovani* found in the macrophages of the reticuloendothelial system; inflammatory cell infiltrates, interstitial edema, and separation of myofibers
	Toxoplasma gondii	H&E	Cysts appear like those in liver (no kinetoplast as in amastigotes of *T. cruzi*)
	Microsporidia	See intestine	See information on intestine
	Plasmodium falciparum (most important), *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi*	H&E	May be marked congestion of the capillaries; blocked with parasitized RBCs and malarial pigment; evidence of anoxia (cloudy swelling, fatty degeneration, myocardial infarcts) may be seen
Skeletal muscle	Protozoa		
	Sarcocystis spp. (rare)	H&E, GMS	Cylindrical cysts (Miescher's tubes) formed (double outer membrane and transverse trabeculae separating the cyst into closed compartments packed with spores); silver stain will clearly reveal the external capsule and septa
	Microsporidia	PAS, AFB, GMS, H&E	Organisms in the genera *Pleistophora*, *Trachipleistophora*, and *Anncaliia* can be found in muscle in compromised hosts (cases on record rare); atrophic and degenerating muscle fibers full of spores (*Pleistophora* in clusters of ~12 organisms); each cluster enclosed by enveloping membrane (pansporoblastic membrane); spores measure 2.8 by 3.4 µm

	Nematode		
	Trichinella spp.	H&E	Diaphragm most commonly invaded; biopsy usually not performed until after day 17, when larvae present a typical, coiled appearance; old, calcified cysts may be difficult to recognize
	Cestode		
	Taenia solium (cysticercosis)	H&E	May also occur in brain, eye, muscles, heart, liver, lungs, abdominal cavity; cyst is oval or round and contains one invaginated scolex with crown of hooklets and four suckers; larva is usually surrounded by tissue reaction capsule, which is wrinkled after tissue processing (very typical appearance, often diagnostic even without cross section of scolex); cysticercus has a dark-staining spiral canal and a scolex surrounded by a thin, lightly staining bladder wall; parenchymatous portion consists of the invaginated scolex with its spiral canal, four suckers, and an armed rostellum with hooks; surface of spiral canal is deeply folded; parenchyma is usually filled with calcareous corpuscles; racemose form of cysticercosis appears as multiple sections of bladder wall tegument (scolex and spiral canal not seen)
Skin and subcutaneous tissue	Protozoa		
	Leishmania spp.	H&E, GMS	See information on liver
	Nematodes *Wuchereria bancrofti*, *Brugia malayi*	H&E	Adults in lymphatic vessels (may be diagnosed in tissue sections); microfilariae in blood
	Loa loa		Adults in subcutaneous tissue; microfilariae in blood
	Onchocerca volvulus		Adults in subcutaneous nodules or in loose connective tissues from deeper areas within the body; may be diagnosed in tissue sections (fibrous nodules); microfilariae in skin; accidental infections with animal species of *Onchocerca* may occur; key features of thick cuticle, transverse ridges, and striations in the middle layer of cuticle are helpful
	Mansonella ozzardi, *M. perstans*, *M. streptocerca*, *Dirofilaria* spp.		Adults in body cavities (abdominal, pleural) and pericardium; microfilariae in blood (*M. streptocerca* in skin)
	Hookworm larvae	H&E	Differentiate from *Strongyloides* spp. (not found in skin)

(continued)

Appendix 34.1 Histologic identification of parasites[a] *(continued)*

Body site	Organisms	Stain	Comments
	Cestodes		
	Spirometra spp. (sparganosis)	H&E	Larvae often invade subcutaneous tissues or muscles (in or about the eyes); acute inflammatory reaction, which is more severe as larva dies and is absorbed; solid-bodied sparganum lacks suckers and surrounding bladder wall seen in other cysticerci
	Sparganum proliferum	H&E	Produces a more severe reaction with metastatic branching and spread throughout the tissues; no scolex seen
	Arthropods		
	Demodex folliculorum (mite)	H&E	Invades hair follicles and sebaceous glands; may be chronic inflammatory cells around mites
	Sarcoptes scabiei (itch mite)	H&E	Superficial skin layer (never below epidermis); preferred site is interdigital spaces, may be elsewhere (heavy infestations, i.e., Norwegian scabies)
	Tunga penetrans (chigoe, sand flea)	H&E	Prefers toes; causes intense itching, even pain
Lymphatic vessels	Nematodes		
	Wuchereria bancrofti, *Brugia malayi*	H&E	Damage ranges from inflammatory sensitization to elephantiasis (tissue hyperplasia)
Testes	Nematode		
	Wuchereria bancrofti	H&E	Worms may be calcified and lymphatic vessel replaced by collagen tissue
Nervous system	Protozoa		
	Naegleria fowleri, *Acanthamoeba* spp., *Balamuthia mandrillaris* (leptomyxid ameba), *Sappinia diploidea*	H&E	Brain lesions demonstrate congestion or hemorrhages, marked cellular infiltration (mononuclear, polymorphonuclear), and abscess formation; trophozoites have large karyosome and no chromatin on nuclear membrane; may be difficult to differentiate *Acanthamoeba* from *Balamuthia*
	Entamoeba histolytica	H&E, PAS	Histolysis of invaded tissue; trophozoite nucleus may not always be visible
	Microsporidia, *Encephalitozoon* spp., *Vittaforma* spp., *Nosema* spp., *Anncaliia* spp., *Trachipleistophora* spp., *Microsporidium* spp.	PAS, AFB, GMS	Spores range from 2 to 4 μm and have been found in cerebrospinal fluid; appearance in tissue similar to other body sites; eye infections reported with *Encephalitozoon*, *Vittaforma*, *Nosema*, and *Microsporidium*

Trypanosoma brucei gambiense, *T. b. rhodesiense*	H&E	May be no reactive changes around organisms (brain); undulating membrane may be difficult to see
Toxoplasma gondii	H&E	See information on liver; there is no cyst formation in an early acute infection (central nervous system more commonly involved)
Plasmodium falciparum	H&E	Congested blood vessels containing parasitized RBCs and malarial pigment
Nematodes		
Angiostrongylus cantonensis	H&E	Produces eosinophilic meningoencephalitis; cross section of worm is characterized by high muscle fibers under the cuticle with two prominent chords; cellular infiltrates composed of eosinophils and mononuclear cells
Toxocara spp.	H&E	Granulomas or necrotic areas within periretinal inflammatory membrane
Cestodes		
Taenia solium (cysticercosis)	H&E	May induce fibrous encapsulation; pronounced cellular reaction may occur as larva begins to die
Echinococcus granulosus	H&E	Wall is finely laminated and has affinity for either acidic or basic dyes
Trematodes		
Schistosoma japonicum	H&E	Hyperemia of meninges overlying granulomas in brain
Paragonimus spp. (any area of brain)	H&E	Cysts may contain eggs, cellular debris, Charcot-Leyden crystals, eosinophils, plasma cells, and lymphocytes; granulomas form around eggs

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[a] Data from references 1–6; not every body site or every parasite is included in this table. AFB, acid-fast bacillus; CNS, central nervous system; GMS, Gomori methenamine-silver stain; H&E, hematoxylin and eosin stain; PAS, periodic acid-Schiff stain; RBC, red blood cell.

Appendix 34.2 General characteristics of helminths

Characteristic	Nematodes	Cestodes	Trematodes	Acanthocephala	Pentastomes (tongue worms)
General	Elongate-cylindrical, bilaterally symmetrical with secondary triradiate symmetry at anterior end; integument nonnucleated cuticula secreted by hypodermis (may be smooth, striated, bossed, or spined); body has 3 main layers (cuticle, hypodermis, somatic muscles); 4 chords arise from hypodermis and project into body cavity; no circular muscles; body cavity is pseudocele (not lined with mesothelium); all viscera suspended in pseudocele; all separate sexes	Adult tapeworm consists of few to many egg-producing units (proglottids) which develop from distal end of scolex; chain called strobila; each proglottid contains male and female genital organs (hermaphroditic); food absorbed through integument; at surface is layer of microvilli (microtriches) over entire outer surface (comparable to brush border of host intestinal epithelium); primitive excretory system; tegument homogeneous and noncellular; circular, longitudinal muscles; proglottids filled with mesenchyme or parenchyma (loose meshwork of branched cells and fluid-filled spaces); calcareous corpuscles usually present in parenchyma	Flukes usually dorsoventrally flattened and hermaphroditic (other than schistosomes); have oral and ventral suckers; integument frequently covered by spines; mesenchyme below integument; circular, oblique, longitudinal muscles; alimentary canal is inverted Y shape; nutrients generally absorbed through tegument	Canals in tegument and arrangement of deeper layers of body wall distinctive; proboscis (retractile into proboscis sheath) armed with spines and body proper; separate sexes; probably related to tapeworms	Body elongate, tongue-like, cylindrical, or moniliform, with many pseudosegments but no separation into head, thorax, and abdomen; head with median subterminal mouth and two pairs of hollow, retractile claws; eyes lacking; sexes separate; striated muscles; have been considered highly modified annelids
Body cavity	Pseudocoelom—yes	No—parenchyma, containing calcareous corpuscles (unique to cestodes, prominent in larval forms)	No—filled with mesenchyme	Pseudocoelom—no; loose parenchymous matrix	Reduced—yes
Body tegument (wall)	Cuticle, hypodermis; cuticle has striations, annulations, or ridges; hypodermis has dorsal, ventral, and lateral chords; well-developed, longitudinally oriented, smooth muscles	Smooth	Smooth or spinous, two suckers (oral and ventral/acetabulum); syncytial epithelium bounded externally by a plasma membrane and internally by trilaminated plasma membrane	Thick, multilayer; outer plasma membrane, thin fibrous layer, thick feltlike middle layer, very thick fibrous inner layer; structure of proboscis with hooks and spines on body surface key features; canals present in tegument	May have spines; cuticle may have prominent spines or may be smooth; subcuticular glands lie below cuticle, as do striated muscle fibers; cuticle contains chitin and has sclerotized openings that are diagnostic for pentastomids

Cuticle (nematodes)	3 layers (cortical, median, basal); surface may have longitudinal ridges (may be called alae); may also have inflations called bosses or plaques	Surface may be covered with hairy projections called microtriches	True cuticle and epidermis shed or fails to develop	NA[a]	NA
Hypodermis (nematodes)	Layer of cells lying between the cuticle and muscles; projects into the pseudocoelom at the dorsal, ventral, and lateral positions to form 4 chords; secretes the cuticle	NA	NA	NA	NA
Chords	Lateral chords most conspicuous; in some groups, lateral secretory/excretory columns lie within or associated with lateral chords	NA	NA	NA	NA
Muscle fibers	Longitudinally oriented, smooth muscles; no circular muscles	Longitudinal and circular smooth muscles	Longitudinal, circular, and diagonal smooth muscles	Longitudinal and circular smooth muscles	Striated muscle fibers
Body muscles	Somatic and specialized				
Somatic (nematodes)	Elongate, spindle-shaped cells; lie in rows under hypodermis, are parallel to and overlap each other; each cell has basal, contractile portion and cytoplasmic, noncontractile portion	Between tegument and subtegumental nuclei are 2 thin layers of muscle fibers; outer layer is circular fibers and inner layer is longitudinal; some differences among species	Outer layer of circular muscle, inner layer of longitudinal muscle; dorsoventral muscle fibers course throughout the parenchyma and surround internal organs	Outer circular and inner longitudinal fibers	Acidophilic glands can be seen in larval sections

(continued)

Appendix 34.2 General characteristics of helminths *(continued)*

Characteristic	Nematodes	Cestodes	Trematodes	Acanthocephala	Pentastomes (tongue worms)
Specialized (nematodes)	Associated with digestive and reproductive systems				
Body cavity contents	Contains digestive tube and tubular gonads	No	No	No digestive system	Yes, intestine large
Digestive system	Mouth, buccal cavity, esophagus, intestine, rectum	No	Oral opening, pharynx, esophagus which bifurcates into a pair of blindly ending tubular intestinal ceca	No—lacunar system; nutrients absorbed through body wall	Simple gut
Reproductive system	Separate sexes; simple tubular structures; usually 1 testis and paired ovaries	Hermaphroditic	Hermaphroditic; 2 testes and 1 ovary; exception is seen with schistosomes (separate sexes)	Separate sexes; 2 testes and 1 ovary, breaks up into "floating" ovaries	Separate sexes
Nervous system	Not relevant for identification	Not relevant for identification	Not relevant for identification	Not relevant for identification	Not relevant for identification
Excretory system	Referred to as secretory/excretory system; varies from large gland cell that opens to the exterior via a duct in ventral midline to being free in the pseudocoelom	Not relevant for identification	Bilaterally symmetrical	NA	NA

[a] NA, not applicable.

Appendix 34.3 Staining characteristics

Characteristic	Comments
Staining action	**Substantive:** Stain that acts immediately and directly on the tissue without the intervention of any other substance **Adjective:** Tissue is first treated with an agent, which in turn attaches the stain to the tissue (mordant staining) **Impregnation:** Involves the deposition of sensitive metallic substances over selected cells and tissue structures that are rendered visible by subsequent reduction of the metal
Staining time	**Progressive:** Using the microscope, performed by watching the degree of staining at various points during the process and stopping the process when the desired staining action has been achieved **Regressive:** Used for the best sharpness of differentiation; whole tissue is stained and then differentiated to remove excess dye from the parts that should be relatively unstained
Action on tissue	**General:** Stain that colors all parts of the tissue equally, providing no significant differentiation **Selective:** Stain that differentiates between classes of tissue or between parts of cells
Differentiation	Using regressive staining methods, the process of removing excess stain is called differentiation; this usually provides sharp staining contrasts because the hydronium and hydroxide ions in the solvents used for differentiation diffuse more rapidly than any dye ion; some of the ways in which a tissue section may be differentiated include the use of acidic or basic medium and excess mordant, buffers, or oxidizers
Staining mechanisms	**Capillarity and osmosis:** Account for penetration of the dye into the interior of the tissue **Absorption or solution:** Passage of the dye molecule from the dye bath solution to the solution in the substance being dyed **Adsorption:** Deposition of dye on the surface of the dyed material (physical surface adsorption plus salt linkages of dye to the protein chain)
Chemical factors	**Acid-base:** Based on the fact that certain cell parts are assumed to be acidic and other parts are alkaline **Ionic strength of dye solution:** Dissolved salt, either neutral or buffered, in the dye solution influences the interaction of dye and tissue; increased ionic strength decreases the staining of both acidic and basic dyes; salt ions may compete with color ion for binding site on the protein molecules **Dye concentration:** Greater amounts of dye are bound with increasing concentrations of the dye; amount of dye bound by tissue is limited by the number of available binding sites **Fixation of tissue:** When cells are fixed, the affinity for stains increases; protein molecules are reorganized so that chemical groups are more available to the dye (characteristic shared by all fixatives); greater basic dye uptake after formalin fixation; more acidic dye uptake after fixation with mercuric chloride fixatives **Temperature:** Increase in temperature increases the diffusion rate of dye molecules; increase also causes protein molecules to swell the fibers, rendering them more open to dye penetration **Staining equilibrium:** Staining is a reversible reaction; when the solution environment is changed, the equilibrium concentration in the tissue is altered

Appendix 34.4 Nuclear and cytoplasmic stains

Stain	Comments
Nuclear stains	
Technical classification	**Reagents used:** Dyes can be either natural or synthetic; natural dyes include cochineal, carmine, and hematoxylin; synthetic dyes include derivatives of substances found in coal tar, oxazines (safranin, basic fuchsin), thiazins (methylene blue, toluidine blue), crystal violets, and gentian violets **How reagents are used:** Depending on mordants, other solutions, etc., results will vary
Nucleic acids	**DNA (nucleus):** Feulgen reaction used to demonstrate DNA (periodic acid-Schiff stain) **RNA (nucleolus, cytoplasm/mitochondria, ribosomes):** Methyl green-pyronin used to demonstrate DNA (methyl green) and RNA (pyronin)
Cytoplasmic stains	
Definition	Usually consist of acidic dyes that contain sulfonic and carboxylic acids that combine with tissue bases, especially those proteins with an excess of the basic amino acids such as arginine, lysine, hydroxylysine, and histidine
Common cytoplasmic stains	**Picric acid:** Nitro dye used as a yellow contrast stain in collagen methods, as well as with hematoxylin **Eosin:** Used as a counterstain with hematoxylin; can be used as an aqueous or alcoholic solution
Mordant	
Definition	A substance capable of combining with a dye and the material to be dyed, thereby increasing the affinity or binding of the dye

Appendix 34.5 Carbohydrate stains

Carbohydrates	Stains[a]	Comments
Polysaccharides	**Iodine:** Starch and by-products (amylose and amylopectin) give blue color; glycogen and amyloid give a red-brown reaction **PAS:** glycogen strongly positive	Glycogen is the only polysaccharide that occurs in higher animals (liver cells, muscle, cartilage, certain parathyroid cells); starch occurs as carbohydrate reserves in plants; cellulose occurs in cell walls of plants
Mucopolysaccharides (acidic mucopolysaccharides, neutral mucopolysaccharides [not very relevant])	**Iodine:** Starch and by-products (amylose and amylopectin) give blue color; glycogen and amyloid give a red-brown reaction **PAS:** For carbohydrates, glycogen is strongly positive, acid mucopolysaccharide does not stain, neutral mucopolysaccharide is not present in significant amounts, but chitin will stain; for lipids, glycolipids and phospholipids are PAS positive; for proteins, pale background color probably due to very small amounts of carbohydrate	Hyaluronic acid is the only example of a simple acidic mucopolysaccharide; main component of ground substance (substance that embeds collagen and elastic fibers) (eye, synovia, some pathologic conditions); for sulfated acidic mucopolysaccharides, heparin is found in a large variety of tissues (tissue mast cells are the site of production), and chondroitin sulfate is found in connective tissue and cartilage
Mucoproteins	**PAS:** Positive **Toluidine blue for mucin:** Positive	Complexes in which the protein, rather than the carbohydrate, is the principal constituent; found in blood group antigens, digestive and respiratory, etc.; the term "mucin" usually signifies mucoprotein
Glycoproteins	**PAS:** Positive	Similar to mucoproteins (hexosamine content, <4%); collagen, reticulin fibers; frequently synonymous with "mucoids"
Glycolipids	**PAS:** Positive	Contain 1 mol each of a fatty acid, sphingosine, and hexose; examples are cerebrosides and gangliosides

[a] PAS, periodic acid-Schiff.

Appendix 34.6 Most likely, secondary, and rare body site locations for human protozoan parasites[a]

Parasite	Life cycle stage	Most likely site(s)	Secondary site(s)	Rare site(s)
Amebae				
Entamoeba histolytica	T	Colon	Liver, lungs, kidneys, skin, small intestine	Brain, heart, lymph nodes, peritoneal cavity, reproductive system, eye, spleen, bone
Entamoeba gingivalis[b]	T	Buccal cavity		
Acanthamoeba spp.	T/C	Eye, brain, skin		Lungs, bone
Balamuthia mandrillaris	T/C	Brain		
Naegleria fowleri	T	Brain		
Sappinia diploidea	T	Brain		
Flagellates				
Giardia lamblia	T	Small intestine, appendix		
Leishmania spp.	A	Skin, liver, spleen, bone marrow		Reproductive system, eye
Trypanosoma cruzi	A	Heart, bloodstream, bone marrow	Muscle, brain, colon, female reproductive system	Stomach, small intestine, lymph nodes
Ciliate				
Balantidium coli	T	Small intestine, appendix, colon		Lymph nodes, peritoneal cavity
Apicomplexans				
Cryptosporidium spp.	CO, O	Small intestine, appendix	Stomach, lungs, liver, gallbladder	
Cyclospora cayetanensis	CO, O	Small intestine		
Cystoisospora belli	CO, O	Small intestine		Lungs, lymph nodes
Sarcocystis spp.	CO	Muscle, heart, small intestine, blood vessel walls		
Toxoplasma gondii	B, Ta	Brain, lungs, heart, lymph nodes, eye, female reproductive system, spleen	Skin, liver, gallbladder, kidneys, pancreas, small intestine, male reproductive system, bone	Bladder, ureters
Plasmodium spp.	M	Bloodstream, red blood cells	Liver	
Microsporidia				
Enterocytozoon bieneusi	S	Small intestine, colon	Lungs, liver, kidneys	
Encephalitozoon intestinalis	S	Small intestine, colon	Lungs, heart, liver, gallbladder, kidneys	
Encephalitozoon spp.	S	Small intestine, colon, eye	Lungs, heart, liver, gallbladder, kidneys, bloodstream vessels, lymph nodes	
Pleistophora	S	Muscle	Eye	
Trachipleistophora	S	Muscle	Eye	
Anncaliia	S	Eye	Vocal cord	
Nosema	S	Eye, muscle (*Nosema*-like)		
Vittaforma corneae	S	Eye		
Microsporidium	S	Eye		
Tubulinosema acridophagus	S	Liver, BAL	Skin	Retina

[a] Adapted from references 1, 5, and 9. A, amastigotes; B, bradyzoites; BAL, bronchoalveolar lavage; C, cyst; CO, coccidian stages; M, malarial parasites; O, oocyst; S, spores and microsporidial stages; T, trophozoite; Ta, tachyzoites.

[b] If found in sputum, can be misdiagnosed as *E. histolytica* from pulmonary abscess.

Appendix 34.7 Most likely, secondary, and rare body site locations for human helminth parasites[a]

Parasite	Life cycle stage	Most likely site(s)	Secondary site(s)	Rare site(s)	Comments
Intestinal nematodes					
Ascaris lumbricoides	A/L	Small intestine (A), lungs (L)	Liver, gallbladder, kidney, pancreas (A)	Pleural cavity	Tendency to migrate if disturbed
Enterobius vermicularis	A	Small intestine, colon	Peritoneal cavity, female reproductive system	Lungs	Extremely common; eggs highly infectious
Trichuris trichiura	A	Colon	Small intestine		"Whipworm"
Capillaria philippinensis	A	Small intestine			Eggs resemble *Trichuris*
Hookworms (*Ancylostoma duodenale*, *Necator americanus*)	A/L	Small intestine (A), lungs (L)			Eggs identical
Trichostrongylus spp.	A	Small intestine			Similar to hookworm disease
Strongyloides stercoralis	A/L	Small intestine (A), small intestine (L)	Lungs, colon (L)	Liver, kidneys (L)	May cause disseminated disease
Tissue nematodes					
Trichinella spp.	A/L	Small intestine (A), muscle (L)		Brain (L)	Pork, bear, walrus meat
Baylisascaris procyonis	L	Brain	Muscle, lungs, heart, liver, gallbladder		Associated with raccoons
Lagochilascaris spp.	A/L	Skin, buccal cavity		Lungs	Rare human infection
Toxocara canis	L	Eye, liver	Lungs	Brain, heart	Causes VLM and OLM
Toxocara cati	L	Eye, liver		Brain, heart	Causes VLM and OLM
Ancylostoma spp.	L	Skin	Muscle	Eye	Causes OLM
Dracunculus medinensis	A	Skin		Pericardium, eye, brain	"Fiery serpent"
Angiostrongylus cantonensis	A	Brain		Lungs, eye	Rat lungworm
Angiostrongylus costaricensis	A	Small intestine, peritoneal cavity			Abdominal disease
Gnathostoma spinigerum	L	Skin, muscle	Brain	Small intestine, eye	Larvae migrate throughout the body
Anisakis spp.	L	Stomach, small intestine		Peritoneal cavity	Associated with sushi and sashimi
Pseudoterranova decipiens	L	Stomach, small intestine		Peritoneal cavity	Associated with sushi and sashimi
Capillaria hepatica	A	Liver			No eggs found in stool
Thelazia spp.	A	Eye			Reported in California
Filarial nematodes					
Wuchereria bancrofti	A	Lymphatics		Lungs, eye	Elephantiasis
Brugia malayi	A	Lymphatics		Lungs	Elephantiasis
Brugia spp. (zoonotic)	A	Lymphatics		Lungs	
Onchocerca volvulus	A	Skin			"River blindness"
Onchocerca spp. (zoonotic)	A	Skin			
Loa loa	A	Skin, eye			African eye worm
Mansonella perstans	A	Peritoneal cavity		Eye	

(continued)

Appendix 34.7 *(continued)*

Parasite	Life cycle stage	Most likely site(s)	Secondary site(s)	Rare site(s)	Comments
Mansonella streptocerca	A	Skin			
Mansonella ozzardi	A	Skin			
Dirofilaria immitis	A	Lungs	Heart	Skin	"Coin" lesions
Dirofilaria repens	A	Skin, eye		Lungs	
Dirofilaria spp.	A	Skin, eye		Bloodstream	
Unusual nematodes					
Oesophagostomum spp.	A/L	Colon	Peritoneal cavity		Nodular mass lesions
Mammomonogamus spp.	A	Lungs, buccal cavity			Y-shaped worms
Dioctophyma renale	A/L	Kidney (A), skin (L)			Primarily right kidney
Tissue cestodes					
Taenia solium (cysticercus)	L	Skin, muscle, brain, eye	Heart		Pork tapeworm
Echinococcus granulosus (unilocular cystic hydatid)	L	Liver, lungs	Muscle, brain, heart, kidneys, eye, spleen, bone	Peritoneal cavity	Sheep-sheepdog cycle; hydatid disease
E. multilocularis (alveolar cystic hydatid)	L	Liver	Brain, lungs	Peritoneal cavity	Difficult to treat
Taenia/Multiceps spp. (coenurus)	L	Skin, muscle, brain, eye	Peritoneal cavity		Most often in central nervous system
Spirometra/ Diphyllobothrium spp. (spargana)	L	Skin, muscle	Lungs, heart, peritoneal cavity, reproductive system, bladder	Brain, eye	Use of raw, infected tissue as poultice
Mesocestoides spp.	L	Peritoneal cavity			Approximately 20 cases
Intestinal trematodes					
Heterophyids	A/E	Small intestine	Heart (E)		Eggs almost identical
Clonorchis/ Opisthorchis	A/E	Liver			Chinese liver fluke
Fasciola	A/E	Liver		Skin, peritoneal cavity	Sheep liver fluke
Paragonimus spp.	A/E	Lungs	Skin, brain (A/E); muscle (A/E)		Lung fluke
Schistosoma mansoni	A/E	Blood vessels, peritoneal cavity, mesenteries (A); colon (E)	Brain (E)	Liver, gallbladder	(E) Inferior mesenteric veins (A)
Schistosoma haematobium	A/E	Bladder, ureters, blood vessels (A); colon (E)			Vesicle, prostate, uterine plexuses (A)
Schistosoma japonicum	A/E	Peritoneal cavity, mesenteries, blood vessels	Lungs (E); brain, small intestine, colon (E)	Liver, gallbladder	(E) Superior mesenteric veins (A)
Unusual trematodes					
Alaria spp.	L	Skin	Stomach, lymph nodes, liver	Eye	Ingestion of poorly cooked frogs (legs)
Arthropods					
Demodex spp.	A/L	Skin (hair follicles)			Follicle mite
Sarcoptes scabiei	A/L	Skin			Itch mite, scabies

(continued)

Appendix 34.7 Most likely, secondary, and rare body site locations for human helminth parasites[a] *(continued)*

Parasite	Life cycle stage	Most likely site(s)	Secondary site(s)	Rare site(s)	Comments
Tunga penetrans	A	Skin			Chigoe, sand flea
Pentastomes	L	Lungs, liver, peritoneal cavity	Lymph nodes, lymphatics in buccal cavity	Eye	Tongue worms
Myiasis (fly larvae)					
Cochliomyia	L	Nose, ear, brain			Infects living tissue; common name is New World screwworm
Calliphora	L	Skin, intestine, GU tract			Infects dead tissue; common name is bluebottle
Cordylobia	L	Skin, usually feet			Infects subcutaneous boils; common name is Tumbu fly
Sarcophaga	L	GI tract, skin, GU tract, vagina			Infects dead or living tissue, depending on species; common name is flesh fly
Dermatobia	L	Skin, boils, brain			Infects living tissues; common name is human botfly
Cuterebra	L	Skin, eye			Infects living tissue; common name is North American botfly
Gastrophilus	L	Skin, migrates to eye			Undergoes "larva migrans" cutaneous migration; common name is horse botfly
Hypoderma	L	Skin, eye, nasopharynx			Undergoes extensive larval wandering (limbs); common name is warble fly
Oestrus	L	Nasopharynx, eye, sinuses			Infects living tissue, resembles conjunctivitis; common name is sheep nasal botfly

[a] Adapted from references 1, 4, and 5. A, adult (for filarial nematodes, includes immature adults, not microfilariae); E, eggs; GI, gastrointestinal tract; GU, genitourinary tract; L, larva(e).

35

Medically Important Arthropods

Arthropods and their relationship to disease
Biological vectors of microorganisms
Bites and envenomation
Tissue invasion
Entomophobia and delusional infestation (parasitosis)
Class Insecta (insects)
Order Diptera (flies, mosquitoes, and midges)
Myiasis
Order Hemiptera (true bugs)
Order Coleoptera (beetles)
Order Siphonaptera (fleas)
Order Anoplura (sucking lice)
Order Mallophaga (biting and chewing lice)
Order Hymenoptera (bees, wasps, and ants)
Order Blattaria (cockroaches)
Class Arachnida (ticks, mites, spiders, and scorpions)
Subclass Acari (ticks, mites, and chiggers)
Subclass Araneae (spiders)
Subclass Scorpiones (scorpions)
Other arthropods
Class Chilopoda (centipedes)
Class Diplopoda (millipedes)
Class Crustacea (copepods, crabs, crayfish, etc.)
Control of arthropods of medical importance
Physical control
Biological control
Chemical control

Over 1 million species in the animal kingdom are found in the phylum Arthropoda. These invertebrates are characterized by the possession of an exoskeleton, an internal hemocele that is filled with hemolymph and the internal organs, and a body that is usually divided into the head, thorax, and abdomen. Arthropods develop through a process called metamorphosis, often involving three or four stages: egg, nymph, and adult (bedbugs, sucking lice, and cockroaches); egg, larva, pupa, and adult (flies, fleas, mosquitoes, and bees); or egg, larva, nymph (one or more), and adult (ticks and mites). Classification of the medically important arthropods is shown in Table 35.1 (1–8).

The importance of this group is due primarily to the morbidity and mortality of the various diseases they transmit, as well as their wide geographic distribution. Arthropods can transmit disease via mechanical transmission, in which the arthropod vector is not a part of the life cycle of the organism, and biological transmission, in which the arthropod vector is an integral part of the life cycle of the organism (multiplication or development or both in the vector). Vector-borne parasitic human infections are listed in Table 35.2. In addition to serving as vectors of various infections, certain arthropods may cause some degree of pathologic changes as a result of their bites, stings, or presence on or in the body. Examples are given in Table 35.3 and Appendix 35.1 of this chapter. Specific diagnostic tests and recommended therapy for the more common arthropods are listed in Appendix 35.2. Specific terms related to the identification of specific arthropods can be found in Appendix 35.3. The relative sizes of some of the more important arthropods can be seen in Fig. 35.1.

Arthropods and Their Relationship to Disease

Biological Vectors of Microorganisms

As biological vectors, arthropods can serve as intermediate hosts for human infections or can accidentally carry microorganisms from one host to another. They can transmit various protozoa (the agents of malaria, leishmaniasis, and trypanosomiasis, and *Babesia* spp.), helminths (filarial worms, lung flukes, and tapeworms), bacteria (*Yersinia pestis* and *Francisella tularensis*), rickettsiae (the agents of typhus, trench fever, and bartonellosis), chlamydiae (the agents of trachoma, lymphogranuloma venereum, and psittacosis), spirochetes (the agents of relapsing fever, yaws, and Lyme disease), and viruses (arboviruses).

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TABLE 35.1 Classification of medically important arthropods[a]

Class	Order	Common name
Insecta	Diptera (true flies)	Blackflies
		Sand flies
		Mosquitoes
		Biting midges
		Deerflies
		Botflies
		Warble flies
		Houseflies
		Tsetse flies, etc.
		Flesh flies
		Blowflies
		Louseflies
	Hemiptera (true bugs)	Assassin bug
		Bedbugs
	Coleoptera (beetles)	Beetles
	Siphonaptera (fleas)	Fleas
	Anoplura (sucking lice; thrips)	Body/head lice
		Crab louse
	Mallophaga (biting/chewing lice)	Dog louse
	Hymenoptera (bees/wasps/relatives)	Honeybees
		Wasps
		Ants
	Blattaria (cochroaches)	German cockroach
		American cockroach
Arachnida	Acari (S)	Hard ticks
		Soft ticks
		Itch mites
		Chiggers
		Follicle mites
	Araneae (S)	Spiders
	Scorpiones (S)	Scorpions
Pentastomida		Tongue worms
Diplopoda		Millipedes
Chilopoda		Centipedes
Crustacea (SP)	Copepoda (S)	Water fleas
	Decapoda	Crabs, crayfish

[a] S, subclass; SP, subphylum.

As vectors, arthropods may serve as mechanical vectors of the etiologic agent, merely transporting the organisms from one host to another or from the environment to a host. However, most agents of arthropod-borne disease use the arthropod as a biological vector, requiring a period of incubation or development in this host. Although most vectors ingest the etiologic agent of disease, they transfer the organisms to humans in several ways, including via an arthropod-derived vomit drop on food or drink or in a puncture wound. Fecal pellets may also contain the infecting organism, as can the hemolymph if the vector is crushed on the skin. Salivary secretions can also contain the infective organisms.

A number of criteria must be fulfilled before an arthropod can be considered a disease vector. The suspected vector must be known to bite humans in a geographic area where human infections are documented. Second, the geographic distribution of the suspected vector must include the distribution of the disease in humans. Third, the causative agent of disease must be isolated from wild-caught vectors and must be the same species as that causing disease in humans. Fourth, it must be shown that naturally or experimentally infected vectors can maintain the infection throughout the complete life cycle of the parasite. Finally, experimental transmission of the parasite by a bite is generally considered proof of the vector's ability to transmit the organism.

Bites and Envenomation

Most individuals have come in direct contact with arthropods which are problems because of their bites and human sensitivity to their saliva or toxins. Human contact occurs when arthropods are feeding or bite in self-defense. Generally these bites do not cause serious problems, unlike stings, for which the reaction may be more severe. The outcome depends on a number of factors, including the arthropod involved, the amount of toxic substance introduced into the body, the physical condition of the bite victim, and possible previous exposure. Individual responses can vary from mild itching and redness to life-threatening reactions.

Envenomation is usually associated with stings; however, some groups can introduce venom during a bite. These include some of the spiders, particularly the widow spiders, violin spiders, and some tarantulas. Arthropods usually associated with the introduction of saliva during the act of biting include the Acari (ticks and mites), Anoplura (sucking lice), Araneae (spiders), Chilopoda (centipedes), Diptera (flies, mosquitoes, and biting midges), Hemiptera (true bugs: bedbugs, kissing bugs, and triatomid bugs), and Siphonaptera (fleas).

Human contact with some groups of urticating caterpillars (which have body hairs that can inject venom) may also produce mild to serious skin conditions (Fig. 34.2 to 34.5). As an example, mature puss caterpillars are covered with gray to brown hairs, under which are stiff spines that are attached to glands that contain poison. When touched, these spines break off in the skin, where they cause severe pain. Eye disease has also been linked to this type of contact (6, 9).

Tick-induced paralysis is also seen and is caused by the injection of toxin from gravid female ticks embedded

TABLE 35.2 Vector-borne human infections

Type of infection and disease	Causative agent	Vector (common name)
Protozoal infections		
Malaria	*Plasmodium* spp.	Mosquitoes
Leishmaniasis	*Leishmania* spp.	Sand flies
Chagas' disease	*Trypanosoma cruzi*	Triatomid bugs
East African trypanosomiasis	*T. brucei rhodesiense*	Tsetse flies
West African trypanosomiasis	*T. brucei gambiense*	Tsetse flies
Babesiosis	*Babesia* spp.	Ticks
Helminthic infections		
Filariasis	*Wuchereria bancrofti*	Mosquitoes
Filariasis	*Brugia malayi*	Mosquitoes
Filariasis	*Dirofilaria* spp.	Mosquitoes
Filariasis	*Mansonella perstans*	Biting midges
Filariasis	*M. streptocerca*	Biting midges
Filariasis	*M. ozzardi*	Biting midges
Onchocerciasis	*Onchocerca volvulus*	Blackflies
Loiasis	*Loa loa*	Deerflies
Dog tapeworm infection	*Dipylidium caninum*	Dog lice, dog fleas, human fleas
Rat tapeworm infection	*Hymenolepis diminuta*	Rat fleas, beetles, grain beetles
Dwarf tapeworm infection	*Hymenolepis nana*	Grain beetles (rare)

in the flesh of the host. A review of 33 cases of tick-induced paralysis in Washington State showed that most of the patients were female (76%) and most cases (82%) occurred in children younger than 8 years; two of the children died (10). Onset of illness occurred over a 3-month period, and nearly all cases indicated probable exposure east of the Cascade Mountains. Of the 28 patients for whom information was available, 54% were hospitalized. When tick identification to species was reported, *Dermacentor andersoni* was consistently reported. Certainly tick-induced paralysis is a preventable cause of illness and death, and if diagnosed early, it requires simple, low-cost intervention (tick removal).

Stinging arthropods can be found in the following groups: Hymenoptera (ants, bees, hornets, and wasps) and the Scorpiones. Individuals vary in their reaction to these stings; anaphylaxis has been seen in cases related to bee stings. The frequency of mass bee attacks has dramatically

TABLE 35.3 Summary of direct effects of arthropods on humans and domestic animals[a]

Condition	Direct/harmful effects	Arthropods
Delusional infestation (parasitosis)	Irrational or destructive behavior	None; often imagined to be skin-invading ectoparasites
Entomophobia/Arachnophobia	Accidents caused by unusual fear of arthropods	Often involve spiders or members of the Hymenoptera (bees, wasps, and relatives)
Physical nuisance	Stress, mental anguish, reduced productivity	Any abundant arthropod that interferes with normal living conditions and/or activities
Contact irritation	Asthma or other respiratory allergies	Any abundant or domestic arthropod that produces airborne allergens
Feeding annoyance and blood loss	Pain, anemia, itching, and other cutaneous allergic reactions; stress, reduced fitness, and death	Temporary and permanent ectoparasites
Envenomization	Prolonged pain, inflammation, cyto/neurotoxic symptoms; anaphylactic allergic symptoms, including death	Arthropods with toxic stings, bites, setae, hemolymph, defensive secretions; usually bees, wasps, ants, spiders, or scorpions
Myiasis	Prolonged pain, tissue and fluid loss, secondary infection, reduced fitness, death	Larval diptera (e.g., *Dermatobia*, *Gasterophilus*, *Hypoderma*, *Oestrus*, *Calliphora*, *Wohlfahrtia*, *Sarcophaga*)

[a] Adapted from references 1–8.

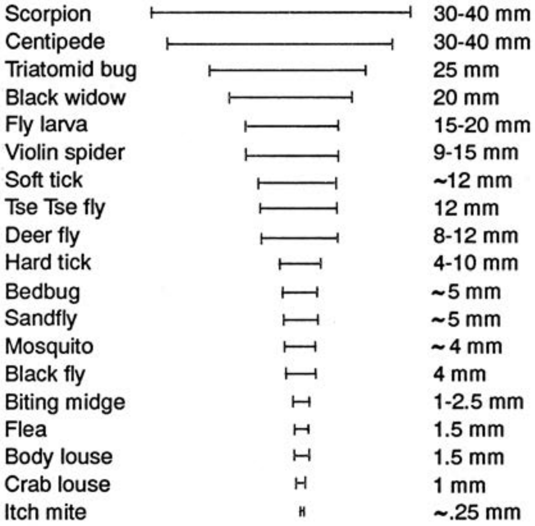


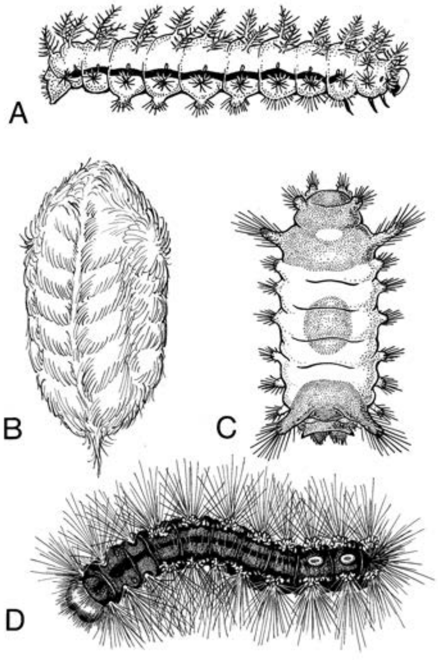
Figure 35.1 Relative sizes of various arthropods. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch35.f1

increased in the Americas following the introduction and spread of the aggressive Africanized "killer" bee (*Apis mellifera scutellata*). Currently there is no specific therapy available. However, an antivenom for the treatment of mass bee attacks has been developed. Venoms from European and Africanized bees appear to be very similar, if not identical (11). Scorpion stings are usually no more severe than bee stings; however, there are exceptions, and each year there are numerous deaths in Mexico as a result of scorpion stings. The venom is thought to be a toxalbumin that affects the nervous and pulmonary systems.

Some millipedes produce secretions that can cause contact vesicular dermatitis. These fluids can be squirted from glands located on the body segments. Blister beetles can also produce these types of fluids, leading to skin blisters within hours after contact.

Although death is rarely attributable to these types of injuries, small children and the very old may demonstrate severe reactions to both arthropod bites and stings.

Figure 35.2 Caterpillars that sting (A and B) or cause dermatitis (C and D). (A) Io moth, *Automeris io*; (B) puss caterpillar, *Megalopyge opercularis*; (C) saddleback caterpillar, *Sibine stimulea*; (D) brown tail moth, *Euproctis chrysorrhoea*. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch35.f2



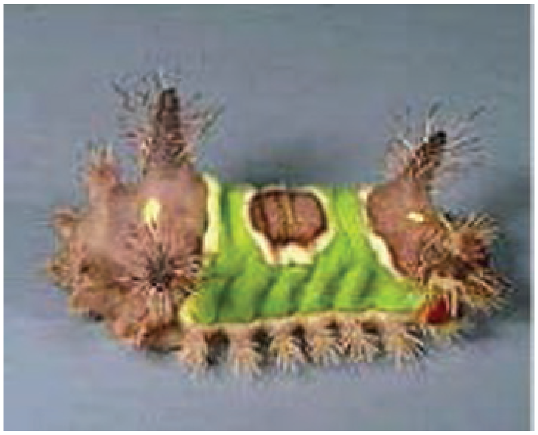
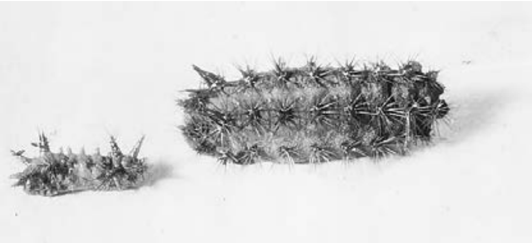


Figure 35.3 Saddleback caterpillar (*Sibine* sp.), which may cause dermatitis and necrosis. The irritant is in the barbed rigid nettling hairs. (Courtesy of R. Bessin, University of Kentucky: http://www2.ca.uky.edu/entomology/entfacts/ef003.asp.) doi:10.1128/9781555819002.ch35.f3

Figure 35.4 Caterpillars: *Monema flavescens* (left) and *Parasa* sp. (right). The small knobs on the body segments carry the stinging hairs, which are too small to be seen in the photograph. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch35.f4



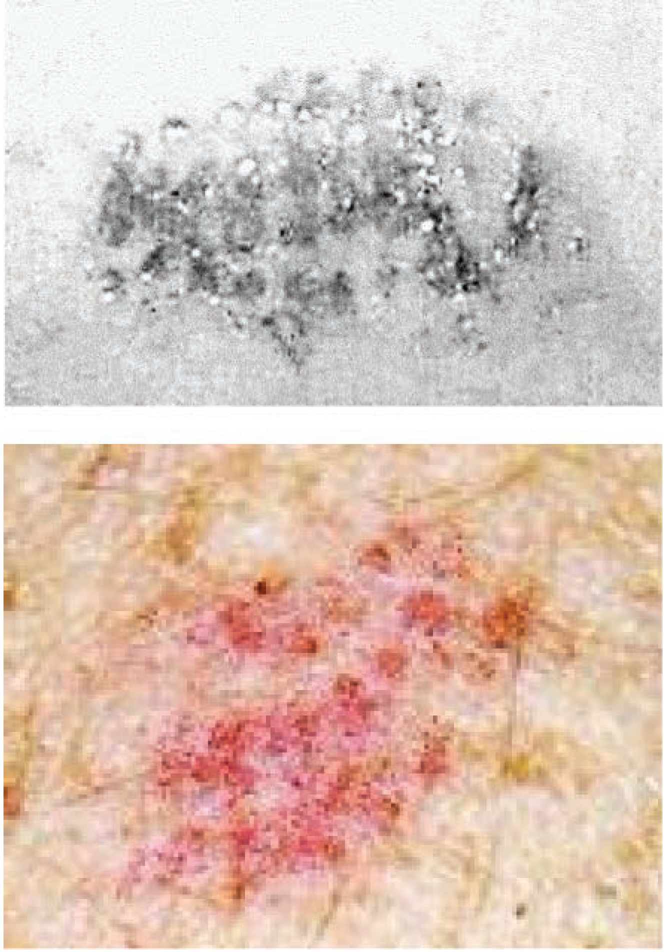


Figure 35.5 *Megalopyge opercularis*, the puss caterpillar. (Upper) Dermatitis is caused by venom introduced into human skin by hollow larval poisonous spines and occurs 24 h after exposure. (Lower) Puss caterpillar sting; note the ecchymosis outline matches the caterpillar's spines and remains visible for several weeks. The initial sting causes intense pain for more than an hour. (From *A Pictorial Presentation of Parasites:* a cooperative collection prepared and/or edited by H. Zaiman.)
doi:10.1128/9781555819002.ch35.f5

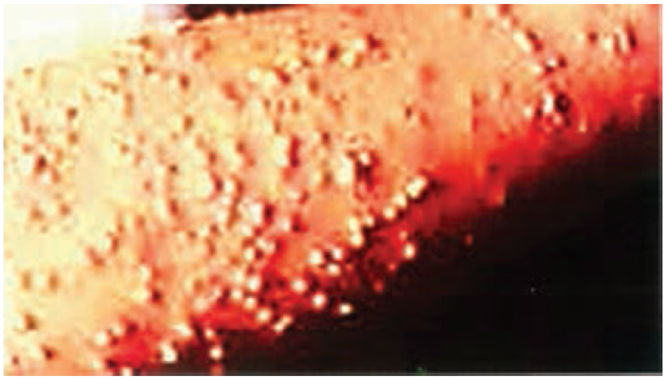


Figure 35.6 Severe reaction to multiple stings from a fire ant, *Solenopsis invicta*, on the arm of a male in Mississippi. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch35.f6

The fire ant, *Solenopsis invicta*, is an example of an arthropod that has proven to be relatively dangerous, particularly in certain patients; the stings cause a fiery, burning sensation (Fig. 35.6). Severe allergic reactions, particularly in children, may require immediate attention. In the southeastern United States, the continued spread of these ants has caused agricultural problems, changes in the ecosystem, and increasing numbers of people with sting sequelae, including hypersensitivity reactions, secondary infections, and rare neurologic problems. Apparently, evolutionary changes have facilitated their expansion northward into Virginia and westward into California; further expansion is anticipated (12). Because of their resistance to natural and chemical control, fire ants can overwhelm their environment, causing destruction of land and animals. They can also cause a variety of health problems in humans, ranging from simple stings to overwhelming anaphylaxis and death. Although deaths caused by imported fire ant stings are rare, they may become more common as the fire ant population expands (13). Two alkaloid components of imported fire ant venom cause cardiorespiratory depression and elicit seizures in rats. Such effects identify these alkaloids as toxic compounds and may explain the cardiorespiratory failure seen in some individuals who experience massive fire ant stings (14).

S. invicta and *Solenopsis richteri* are not the only ant species to cause serious allergic or adverse reactions. Ant species belonging to six different subfamilies (*Formicinae*, *Myrmeciinae*, *Ponerinae*, *Ectatomminae*, *Myrmicinae*, and *Pseudomyrmecinae*) and 10 genera (*Solenopsis*, *Formica*, *Myrmecia*, *Tetramorium*, *Pogonomyrmex*, *Pachycondyla*, *Odontomachus*, *Rhytidoponera*, *Pseudomyrmex*, and *Hypoponera*) have this capability. Awareness that species other than imported fire ants may cause severe reactions should lead to more rapid patient evaluation and treatment (15).

Tissue Invasion

A number of arthropods are capable of actual tissue invasions, some of which may occur in the superficial tissues and some in deep body sites. These groups include Acari (ticks and mites), Diptera (flies, mosquitoes, and biting midges), Pentastomida (tongue worms), and Siphonaptera (fleas). The pentastomes are wormlike parasites that are related to the arthropods. On ingestion of eggs by humans, larvae migrate to the tissues, where they develop into nymphs and become encapsulated. Although most of these infections are asymptomatic, clinical manifestations can occur and include inflamed and enlarged lymph nodes, abdominal pain, jaundice, and ocular lesions (see chapter 29).

Various ticks, mites, and fleas tend to invade tissues at the superficial levels; these include *Sarcoptes scabiei* (itch mite), *Tunga penetrans* (chigoe flea), and members of the *Argasidae* (soft ticks) and the *Ixodidae* (hard ticks). Not only do these arthropods cause painful bites, but also they are responsible for transmission of diseases caused by bacteria, viruses, and rickettsiae.

When we consider arthropod tissue invasion, we usually think of myiasis, i.e., infestation with fly larvae (maggots). Clinically, myiasis is often categorized according to the part of the body invaded: cutaneous, mucocutaneous, intestinal, and genitourinary. Some larvae develop only in living tissue, while others prefer dead or necrotic tissue. Entry sites for egg or first-stage larva deposition can include unbroken skin, open wounds or lesions, nose or ears, mouth with subsequent swallowing, and genitourinary openings. There are three general groups of myiasis-causing flies: (i) those that require a host for the development of their larval stages, (ii) those that develop in a host if entry occurs via wounds or sores but development in a host is not required, and (iii) those that accidentally invade a host but generally complete larval development without a host.

Entomophobia and Delusional Infestation (Parasitosis)

Fear of insects, entomophobia, is sometimes seen and may become an issue in mentally disturbed individuals (1). These patients may report insect infestations, describing in great detail the presence of tiny insects in or on the skin. Symptoms may be described as crawling or biting sensations and are often accompanied by excessive scratching by the patient.

Delusional parasitosis usually represents a more serious emotional disorder in which patients believe that they have parasites in or on the body. It is also called the Ekbom syndrome after the Swedish neurologist Karl Axel Ekbom, who did extensive work on this syndrome. This name, however, is ambiguous and not recommended, because it is also used to refer to restless legs syndrome.

Delusional parasitosis can be primary, secondary, or organic. Primary delusional parasitosis consists of a single delusional belief of having been infested by a parasite and comes under monosymptomatic hypochondriacal psychosis. Secondary delusional parasitosis can occur in the context of other mental disorders like schizophrenia, depression, and dementia. Organic delusional parasitosis occurs secondary to organic illness, such as hypothyroidism, vitamin B_{12} deficiency, diabetes, cerebrovascular disease, cocaine intoxication, HIV infection, allergies, and menopause. These patients often describe many physician visits and consults with unproductive results; laboratory findings are consistently negative. They are absolutely convinced that they have legitimate problems and continue to try to get confirmation of problems that are imaginary rather than real. They tend to bring in small bits and pieces of skin or debris, pictures, drawings, etc., none of which demonstrate true infestation. Often, these bits and pieces of debris are of nonanimal origin (string, paper, plant material, etc.). We have received complete notebooks filled with 8-by-10-in. photographs of “parasites”—none of which depict anything that can be recognized as possible arthropods or any other type of parasite. Morgellons, which refers to cutaneous symptoms like biting, crawling, or stinging sensation, finding fibers on or under skin, and persistent skin lesions, is often seen in delusional parasitosis (16).

In recent years, patients have reported the presence of specific parasites less commonly, and the name delusional parasitosis thus has the disadvantage of covering only one group of pathogens. The broader term “delusional infestation” has been recommended as the future designation. This name highlights the core psychopathological feature (a thought disorder) and covers all kinds of (even “newly emerging”) imaginary pathogens by referring to the delusional theme “infestation” and not to a single species.

These patients search out specific medical professionals, often by making multiple telephone calls and calling medical and other scientific societies. Unfortunately, they can benefit only from psychiatric treatment. However, patients with delusions of parasitosis generally reject psychiatric referral. The diagnosis of delusions of parasitosis can often be made on the basis of the history alone, but it is important to ensure that the patient does not have an organic skin disorder and that the delusion is not secondary to another mental or physical illness.

Specimens are usually presented in a small bin, vessel, bag, piece of paper, or plastic foil to protect the “pathogens.” Usually, the patients handle these allegedly dangerous pathogens without disgust or anxiety. Instead, they are collected and stored like trophies. The “pathogens” usually consist of materials, such as dander, crusts, scabs from healing skin lesions, hair, threads and other particles from clothes, fibers, dirt, sand, arthropod parts, or breadcrumbs. More and more patients present digital photos or movies.

Patients ask the physician for an extensive diagnostic work-up, no matter how many previous dermatological and microbiological tests were unremarkable with negative results. These patients will not be “relieved” by negative findings. Instead, “nonfindings” will be interpreted as “incompetence” of the doctor, or patients will find another explanation to maintain their beliefs (e.g., “the sample was taken at the wrong site or the wrong time; a bad microscope, wrong medium, or wrong analytical method was used; a mix-up of samples, names, or reports occurred,” and others). Many patients request additional therapy rather than more diagnostic testing. They “already know” what they have and often ask to be prescribed the most aggressive antibiotic, virucide, pesticide, or other medication to get rid of the pathogens.

In spite of these psychiatric problems, it is still appropriate to check all possibilities: bites from unrecognized mites or other insects, either at home or in the workplace, or potential exposure to other allergic stimuli that may be unrelated to insect or organism contact needs to be considered. Unfortunately, in many of these patients, the presentation is so convincing that it is difficult at first to recognize the situation as a mental rather than actual physical problem. For adequate therapy, interventions at all steps of the cycle are required, including local skin therapy to reduce itch, the prevention of further manipulations, and sufficient antipsychotic treatment of the fixed belief.

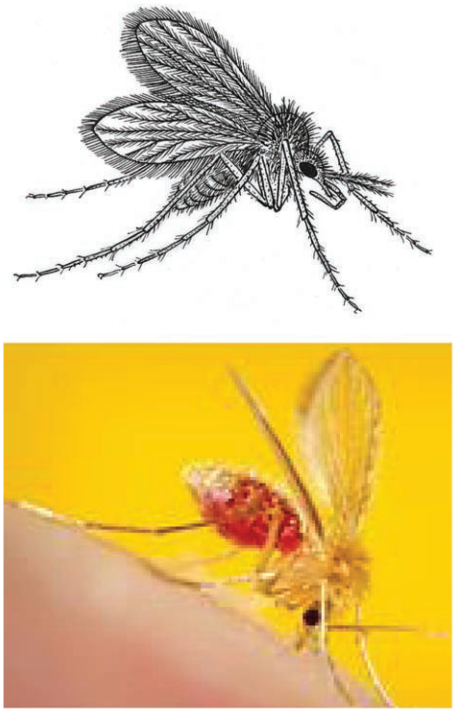


Figure 35.7 Sand fly (*Phlebotomus* and *Lutzomyia* spp., vectors of *Leishmania* spp.), ~5 mm. (Upper, illustration by Sharon Belkin; lower, color image courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch35.f7

Class Insecta (Insects)

Some of the more important orders are presented in this section. Adults have a segmented body composed of the head, thorax, and abdomen; one pair of antennae; three pairs of legs; and, in some cases, wings. The exoskeleton is composed of hard plates (sclerites) separated by flexible membranes (conjunctivae or arthrodial membranes) or grooves (sutures).

Order Diptera (Flies, Mosquitoes, and Midges)

The order Diptera contains numerous kinds of flies, mosquitoes, and midges. The members of this group have two pairs of wings, one pair of which, the halteres, are vestigial and are used for balance only.

Sand Flies (Leishmaniasis)

Species of the genus *Leishmania* (Kinetoplastida *Trypanosomatidae*) are the causative organisms of leishmaniasis, and these parasitic unicellular protozoans are usually transmitted between vertebrate hosts by the bite of bloodsucking female phlebotomine sand flies. Sand flies occur mainly in the tropics and subtropics, limited to areas where the temperatures are above 15.6°C for a minimum of 3 months of the year. They are found in a number of habitats, from below sea level to 2,800 m above sea level or higher. Sand flies are small, long-legged flies that are about 5 mm long (Fig. 35.7). These small flies are able to pass through ordinary mosquito netting. Two genera (*Phlebotomus* in the Old World and *Lutzomyia* in the New World) are of medical importance. Only the females feed on blood, usually that of mammals; such feeding normally occurs at night. The bite site is usually indurated and inflamed, with a wheal 1 to 2 cm in diameter, and is accompanied by pruritus. Some patients even respond with a systemic allergic reaction, including fever, nausea, and malaise. Although many sand flies can fly extended distances, they usually move in short, jerky hops along walls and ceilings. Spraying with residual insecticide is somewhat effective; however, elimination of larval habitats would be a more important control measure.

Sand flies are a geologically old group, being identified from about 120 million years ago, prior to the origin of mammals. They probably originally fed on reptiles, and their ancient status led to the evolution of different groups in the New and Old Worlds. Their main importance is as vectors of *Leishmania* spp., causing human cutaneous, mucocutaneous, and visceral disease. About 80 of 700 known sand fly species are known vectors of *Leishmania* spp. Two species of *Lutzomyia* occur in south and central Texas, as well as parts of Mexico, and are probably the vectors of cutaneous leishmaniasis in Texas. It is interesting that lectins and toxins in the plant diet of *Phlebotomus papatasi* can kill *Leishmania major* promastigotes in the sand fly and in culture; these vegetation components may decrease parasite transmission in certain areas (17). Control measures against the adult flies include the use of the bacterial insecticide *Bacillus thuringiensis* (18).

Sand flies are small (<5 mm long) and fragile. These tiny, densely haired flies tend to rest with their wings held upward at 60°. This resting appearance, along with a dark-gray to dusty-brown color, large eyes, mouthparts at least as long as the head, long antennae, long palpi, and a fuzzy appearance, provide excellent clues for identification. Females have a plump, cylindrical abdomen, somewhat tapered distally, which appears to end with two rounded, paddle-like segments (cerci). There are a total of six genera, *Phlebotomus*, *Sergentomyia*, and *Chinius* in the Old World and *Lutzomyia*, *Brumptomyia*, and *Warileya* in the New World; *Phlebotomus* and *Lutzomyia* are the two most common genera. In addition to sugars from plants, females require one or more blood meals for each batch of eggs. Sand fly bites tend to be quite painful. The use of insecticides, protective clothing, and clothing/bednets treated with permethrin will reduce contact with sand flies. In arid regions of the Middle East that are inhabited by colonial rodents, deep plowing over concentrations of burrows tends to reduce *Leishmania major* reservoirs and vector habitats (5).

There is evidence of an increasing range of sand fly species, especially for the Atlantic Coast and inland parts of Germany, Switzerland, and Austria. The most important and urgent necessity within the community of entomologists working on phlebotomines is the need to record the extremes of distribution of each species and obtain data on their regional presence/absence along with increased sharing of the data throughout European projects.

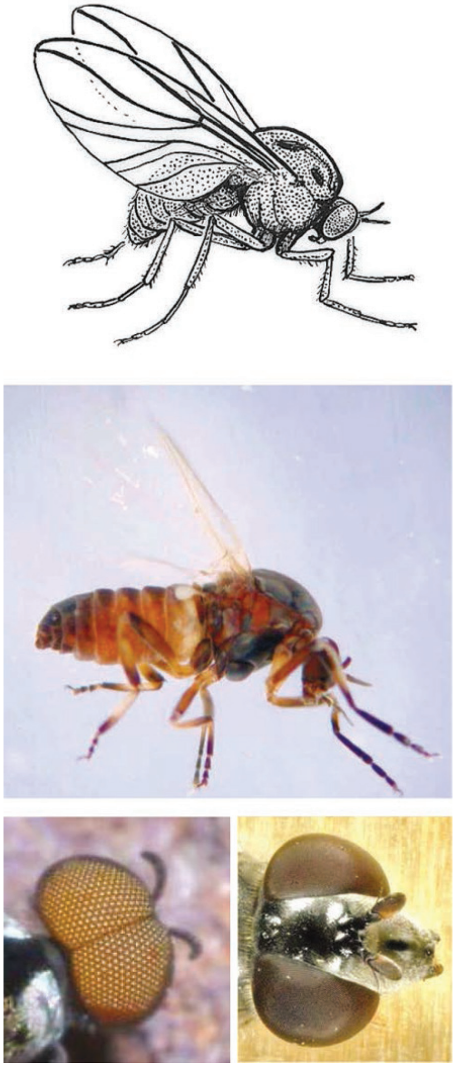


Figure 35.8 Blackfly (*Simulium* sp., vector of *Onchocerca volvulus* and *Mansonella* spp.), ~4 mm. (Top, illustration by Sharon Belkin; middle, color image courtesy of A. Renz, Programme Onchocercosis, http://www.riverblindness.eu/onchocerciasis/simulium-vectors/.) (Lower, left) Eye structure of a *Simulium* male (holoptic); (right) eye structure of a blackfly female (dichoptic). (From *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman.)
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Blackflies (Filariasis [*Onchocerca* and *Mansonella* spp.])

Blackflies (buffalo gnats, turkey gnats, Kolumbtz flies) transmit *Onchocerca volvulus* worldwide; in the Old World these vectors are limited to Africa, while in the New World the vectors are found in various parts of Central and South America. Blackflies are small flies (*Simulium* spp.) measuring 1 to 5 mm long and usually having a stout body and humped back (Fig. 35.8). Only the females suck blood, and swarms may attack humans as well as domestic and wild mammals, usually during the daytime. The blood meal is required for egg development. Although not usually that painful, the bite is accompanied by intense itching and the development of a raised, ulcerative lesion at the site, most probably caused by the saliva in the puncture wound. Individuals who are particularly susceptible may experience severe pruritus and body swelling (blackfly fever), which is seen predominantly in children in rural areas of the northeastern United States. Similar to Hymenoptera, a Simuliidae bite occasionally results in extreme hypersensitivity and should be considered in appropriate cases.

These flies normally do not bite indoors and may fly long distances from their habitats. A massive control effort is now under way in West Africa to lower the intensity of *Onchocerca* transmission to a level of infection that does not lead to visual impairment. Because flight distances are long and the larvae develop in water, control measures are difficult at best. Simuliids breed in running water, ranging from torrential mountain streams to slow-moving lowland rivers, and a few species adapt to streams with little perceptible current. Development occurs in fast-running streams, often very deep; one species was found breeding at depths down to 190 ft (ca. 50 m). Adult appearance occurs predominantly in the daytime, depending on light and temperature. In *S. damnosum* 60–90% of the day's activities occur by midday and there is no emergence at night.

Simuliids have to survive periods when the temperature is too low to sustain routine activities and when the rivers dry up during the off season. They can apparently reduce their respiratory rate and replace trehalose in the hemolymph by high polyhydric alcohols, thus remaining dormant below 4°C. Once the temperature rises, the reverse process is rapidly completed and growth resumes. These flies may not be present in sufficient numbers to be pests, but they can still be important vectors of the filarial worm causing onchocerciasis or "river blindness."

The biting preference of *Simulium* vectors (buffalo gnats) has been known to influence the distribution of *Onchocerca* nodules and microfilariae in the human body. In Africa, *S. damnosum and S. neavei* have been reported to tend to bite in the ankle region, thus resulting in a higher density of nodules and microfilariae in the lower part of the body, whereas in central America, *S. ochraceum* bites mostly in the head and neck regions, producing a higher density of nodules and microfilariae in the upper body.

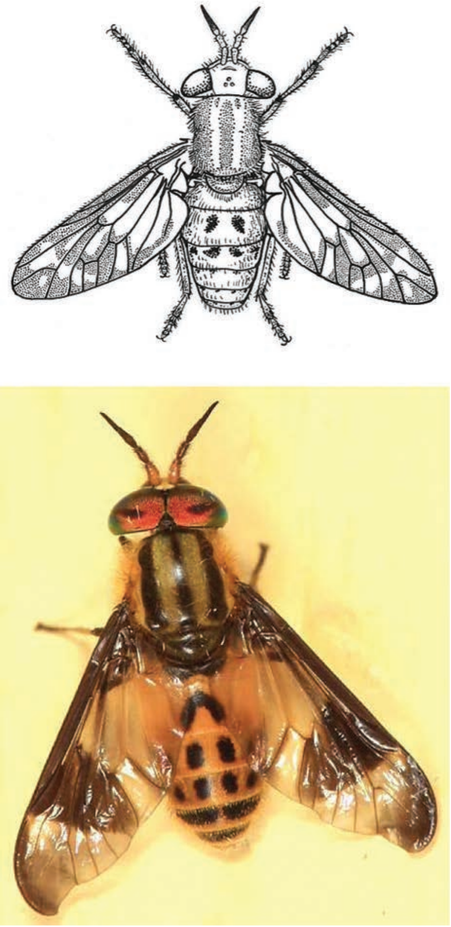
In Brazil, black fly saliva was determined to be responsible for pemphigus foliaceus, also known as bulbous autoimmune disease or wild fire disease. It was also named as the cause of death in immigrants suffering from Altamira hemorrhagic syndrome, which is characterized by hemorrhage of the skin around the bites (multiple petechiae and ecchymosis), bleeding mucosa, and anemia. Female black flies exhibit pool feeding behavior, in which salivary proteins injected at the injury site maintain the blood meal in a fluid state. These proteins include anticoagulants, vasodilators, and modulators of platelet aggregation (19).

The adult *Simulium* is characterized by a small, rounded body, bead-like antennae, an arched thorax, and a pair of wings that span 6 to 10 mm with thickened veins near the leading edge. Most flies are black in color but some species may be orange or yellow. The male's eyes are holoptic (meeting along the dorsal length of the head) (Fig. 35.8), while the female's eyes are dichoptic (separated by an anterior segment called the frons). Ventral on the head is the mouth, a complex structure marked by two maxillary palps attached near the base of the proboscis. The labium forms the back of the proboscis and envelops the other parts of the mouth, which include the serrated mandibles and the toothy posterior maxilla called the laciniae. The color patterns of the legs and thorax can be useful in species identification of *Simulium*.

Deer Flies (Filariasis [*Loa loa*])

Adult flies (*Chrysops* spp.) are medium sized to large (about the size of a housefly or larger) and stoutly built, with large eyes and dull-colored bodies (Fig. 35.9). Only

Figure 35.9 Deerfly (*Chrysops* sp., vector of *Loa loa*). (Upper) Female, 8 to 12 mm (illustration by Sharon Belkin). (Lower) Male, color image (courtesy Tom Murray, http://wiki.bugwood.org/File:Chrysops_tom_murray.JPG).
doi:10.1128/9781555819002.ch35.f9



the female is adapted to take a blood meal. Adult males and females will take nectar, and some are specially adapted for drinking from deep flowers by using a long proboscis. However, most females use their blade-like mandibles to slice into skin, drinking the blood that pools in the cut. The bite site can become a painful wound that takes a long time to heal; individuals may also respond with an allergic reaction to the saliva of the fly. A person can become increasingly sensitive to repeated bites.

Day-biting flies transmit the human form of disease with *L. loa*, a disease that affects up to 13 million people. This biting time preference coincides with the peak microfilaremia in humans. The adult flies live high in the forest canopy and descend to the forest floor to feed on humans. While most blood-sucking insects are repelled by wood fire smoke, apparently in Africa the flies are attracted to wood fires. Control measures have not been particularly successful, and loiasis continues to be a significant problem in West Africa.

Deer flies are slightly larger than house flies and mostly yellow or black with darker stripes on the abdomen and dark markings or patterns on the wings. They have thick, elongate antennae and brilliant green or golden eyes with zigzag stripes.Males are holoptic (eyes large and touching) while females are dichoptic (normal eyes). All have a distinct wing venation where the R4+R5 vein encloses the tip of the wing. Characters on the face and coloration/patterns may be helpful in identifying these flies to the species level.

Tsetse Flies (African Trypanosomiasis)

An estimated 60 million people are at risk for African trypanosomiasis, with 300,000 to 500,000 new cases each year, primarily in the Democratic Republic of Congo, Angola, and Sudan. Tsetse flies (*Glossina* spp.) are robust, honey-brown flies measuring 6 to 15 mm long (the size of a housefly) (Fig. 35.10). The genus habitat is limited to the African continent south of the Sahara. Both sexes are voracious blood feeders, and both can transmit parasites. Adult flies of the important vectors are daytime feeders and are attracted to moving animals and humans. The flies use movement and smell to find appropriate hosts. Although they can fly long distances, their flight pattern is generally limited to the closest available host and back to particular vegetation, where they spend long periods of rest. The wings have the characteristic "hatchet" cell, which is cleaver shaped (Fig. 35.10).

Tsetse flies are diurnal and only active between the temperatures of 18 and 32°C. They feed primarily on suids and bovids, but blood from animals as diverse as monitor lizards and hippopotami has been found in their guts. There is no sexual dimorphism in their feeding habits, except that the female must eat more to sustain the large larvae during pregnancy. They respond to CO_2 produced by the exhalation of animals. Both sexes feed exclusively on blood and produce anticoagulants to prevent clotting. They find their targets through sight and smell. They suck blood every 2 to 3 days, and each blood meal lasts 1 to 10 minutes. Flies are active only for about 1 hour a day. In the meantime they rest in the surrounding vegetation.

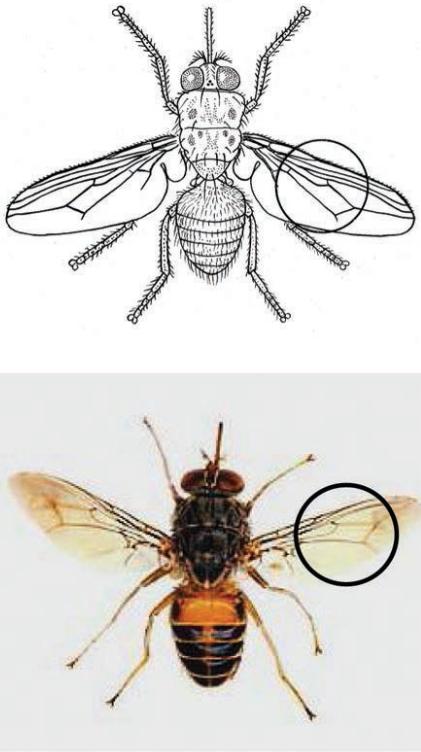


Figure 35.10 Tsetse fly (*Glossina* sp., vector of African trypanosomes). (Upper) Female, ~12 mm. The characteristic "hatchet" cell is seen within the circle. (Illustration by Sharon Belkin.) (Lower) Color image (courtesy of the Universidade Federal do Rio Grande do Sul, Faculdade de Veterinaria, Daniele Focosi, http://www.ufrgs.br/imunovet/molecular_immunology/sitemap.html). doi:10.1128/9781555819002.ch35.f10

Adult females do not lay eggs but are larviparous, i.e., the eggs hatch inside the uterus, where they are nourished with the secretions of a specific gland. There is always only one larva developing in the uterus. It needs about 10 days to reach maturity (stage III). Then the female deposits the larva on dry soil below the vegetation. The larva crawls into the soil and pupates without further feeding. The pupal stage lasts 3 to 13 weeks. An adult fly lives for about 3 months. There are about four generations a year.

The adults are long-lived and fly long distances, usually along a preferred flight line; they live in areas called fly belts. Some species live by rivers, and others are found in the savanna. They feed on many animals as well as humans. This fact, along with their long life span, makes vector control difficult; it sometimes involves such drastic measures as killing off game animals in close proximity to humans or moving whole villages out of these fly belts. The use of insecticides has also proven to be somewhat successful. Wherever there are tsetse flies, it is impossible to keep cattle without regular chemotherapy. Trypanosomiasis has inhibited or totally prevented the development of a cattle-raising industry in much of tropical Africa. Currently, cattle ranches are still impractical in the savanna areas of Africa. Also, there is increasing resistance to melarsoprol, the key drug for central nervous system involvement in African trypanosomiasis.

Stableflies, Houseflies, and Horseflies (Potential Carriers of Infectious Organisms)

Stableflies. Flies in the genus *Stomoxys* (stableflies) (Fig. 35.11) resemble the housefly. Both male and female flies can be tremendous pests, with an impressive bite (causing a stabbing pain) which involves ripping open the skin, through which the biting apparatus is inserted. They measure about 6 or 8 mm long and have four distinct, dark longitudinal stripes on the thorax and several dark spots on the abdomen, with sharp mouthparts protruding from the head. Along the southeast coast of the United States, these flies breed in decaying seaweed and can be a tremendous tourist problem during fly season. There is low host specificity, although they feed mainly on the blood of cattle and horses. Adults locate a host by sight, and feeding is usually completed in 2 to 5 minutes.

Although control of the larvae has been difficult, insecticides are effective against the adult flies. Residual insecticides are initially effective, but these flies rapidly develop resistance. The stablefly breeds in a number of habitats commonly found in agricultural areas such as decaying straw, oats, rice, barley, wheat, silage, horse manure, lot manure (manure from pig farms), and cow manure.

Figure 35.11 Stablefly (*Stomoxys* sp.), 6 to 15 mm long. (Left photograph courtesy of Duane J. Gubler, Centers for Disease Control and Prevention; right image courtesy of Jim Kalisch, University of Nebraska-Lincoln, Dept of Entomology, http://entomology.unl.edu/images/muscidflies/.)
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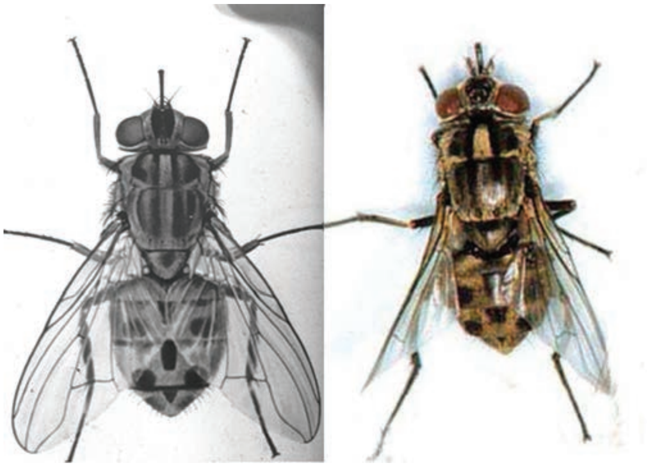

Houseflies. Houseflies can be easily identified by the four dark, longitudinal stripes on top of the thorax, or middle body region. They vary in length from one-eighth to one-fourth of an inch. Their mouth parts are adapted for sponging up liquids; they cannot bite and can only ingest liquid food. They feed on solid food by regurgitating saliva on it. The saliva liquifies the solid material which is then sponged up with the proboscis. These flies require water since they are continually salivating and voiding liquids. Fly specks seen on many surfaces visited by houseflies are the excreted wastes. Houseflies can harbor >100 different pathogens, most of which can be transmitted. They range from viruses to helminths and include protozoan cysts, oocysts, spores, and helminth eggs. There are several ways in which houseflies can disseminate pathogens. The surface of the body can be contaminated; pathogens can be regurgitated onto food through the fly's vomit drop; or pathogens can pass through the gut of the fly and be deposited in its feces.

Horseflies. Horseflies are blood-sucking insects that can be serious pests of cattle, horses, and humans. Horseflies range in size from three-quarters of an inch to 1 1/4 inches long and usually have clear or solidly colored wings and brightly colored eyes. Female horseflies are active during the day. They are attracted to such things as movement, shiny surfaces, carbon dioxide, and warmth. Once on a host, they use their knife-like mouthparts to slice the skin and feed on the blood pool that is created. Bites can be very painful, and there may be an allergic reaction to the salivary secretions released by the insects as they feed. Like mosquitoes, female horseflies must ingest a protein-based blood meal before they can reproduce. Most horseflies will avoid people who are using insect repellent. A high-quality insecticide sprayed on the body or around the nearby area usually wards off these insects. Repellents containing diethyltoluamide or DEET are particularly effective.

It is interesting that in areas such as northern Africa where tsetse flies are not found, domestic animal trypanosomes, *T. evansi* and *T. vivax vivax*, are transmitted mechanically by horseflies (*Tabanus*) and stableflies (*Stomoxys*). These flies can spread trypanosomes within domestic animal herds, causing epidemics with tremendous financial loss.

Biting Midges (Filariasis [*Mansonella* spp.])

Biting midges (*Culicoides* spp.) are the smallest of the biting flies, measuring between 1 and 4 mm (Fig. 35.12). They are also known as "punkies," "no-see-ums," "gnats," or

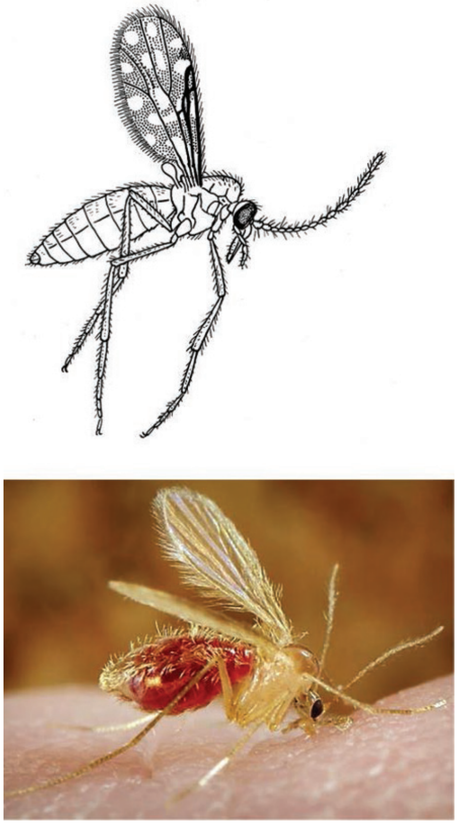

Figure 35.12 Biting midge (*Culicoides* sp., vector of *Mansonella* spp.), 1 to 2.5 mm (usually 1 to 4 mm long). (Upper, illustration by Sharon Belkin, adapted from Faust EC, Russell RF, June RC [ed], *Clinical Parasitology*, 8th ed, Lea & Febiger, Philadelphia, PA, 1970; lower, color image courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch35.f12

"flying teeth." Only the females feed on blood, and swarms may attack humans or other mammals, usually at dusk, when the air is still. The bites of biting midges inflict a burning sensation and can cause different reactions in humans, ranging from a small reddish welt at the bite site to local allergic reactions that cause significant itching. Screen and netting controls are difficult because of the small size of the adult fly.

Male and female biting midges feed on plant sap and nectar, the primary energy sources for flight and for increased longevity of females. Egg production requires a protein source, which is obtained either from the body fluids of small insects or from vertebrate blood. Male biting midges are not attracted to vertebrates, and their mouthparts are not capable of biting.

The genus *Culicoides* is widely distributed throughout the world and ranges from sea level to almost 14,000 ft in Tibet. Members of the 1921 Everest expedition complained of being bitten continually from base camp almost to the summit. These biting midges can cause acute discomfort, particularly on warm, humid days; in spite of their small size, the bite often produces a severe local reaction. Females make small cuts in the skin, and the saliva contains an anticoagulant. Once the blood pools, it is sucked up. It was calculated in the early 1980s that because of the tremendous number of bites to which a human can be subjected, a person spending 1 h per day on the beach in Trinidad in the early morning would receive 38 bites infective for *Mansonella ozzardi* in the course of a year. However, with the tremendous tourist industry impact, control measures are constantly being revisited.

Success in suppressing adult biting midges with insecticides is somewhat limited and only under certain conditions. The installation of window and door screens consisting of a mesh size smaller than normal window screen is much more effective in reducing bites because such screens prevent female biting midges from entering dwellings. Similarly, people camping in certain areas of the western United States should use tents outfitted with "biting midge screening" that consists of a mesh size smaller than normal window screening.

Mosquitoes (Malaria, Filariasis, and Arbovirus Infections)

Mosquitoes are slender, delicate insects that are distributed worldwide. There are over 2,500 different species, of which 150 species occur in the United States. All mosquitoes require water in which to complete their life cycle, which can vary in quality from melted snow water to sewage effluent and can be in any container imaginable. The type of water in which the mosquito larvae is found can be an aid to the identification of which species it may be. Also, adult mosquitoes show a very distinct preference for the types of sources in which to lay their eggs, e.g., such places such as tree holes that periodically hold water, tidewater pools in salt marshes, sewage effluent ponds, irrigated pastures, rainwater ponds, etc. Each species therefore has unique environmental requirements for the maintenance of its life cycle.

Only the adult females bite humans and other animals. The male mosquitoes feed only on plant juices. Some female mosquitoes prefer to feed on only one type of animal while others can feed on a variety of animals. Female mosquitoes feed on humans, domesticated animals, all types of birds including chickens, all types of wild animals, and various reptiles. The life span of the adult mosquito usually depends on several factors: temperature, humidity, sex of the mosquito, and time of year. Most males live about a week and females live about a month (Fig. 35.13). When biting with their proboscis, they stab two tubes into the skin: one to inject an enzyme that inhibits blood clotting, the other to suck up the blood.

Some species deposit eggs singly; some eggs remain single, while others are stuck together in rafts of 200 to

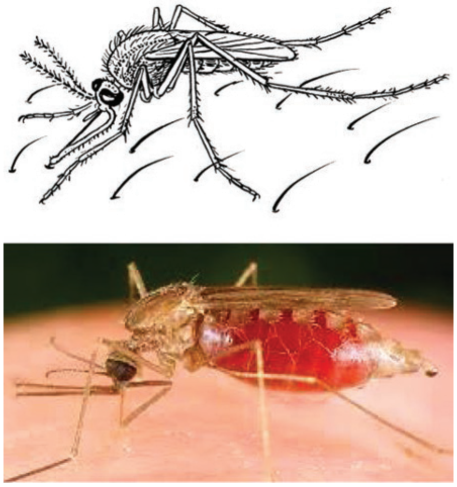

Figure 35.13 (Upper) Mosquito, ~4 mm (illustration by Sharon Belkin). (Lower) Female *Anopheles freeborni* mosquito taking a blood meal (courtesy of the CDC Public Health Image Library).
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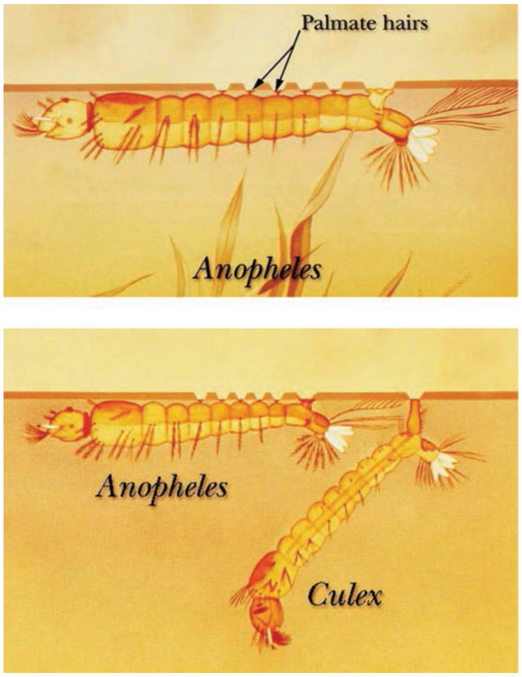

Figure 35.14 Mosquito larvae. (Upper) *Anopheles* must lie parallel to the water to obtain oxygen through a breathing opening. (Lower) *Culex* has a breathing tube, so that it lies almost perpendicular or at an angle to the surface of the water. Note other physiological differences between *Anopheles* and *Culex*. (Courtesy of the CDC Public Health Image Library.)
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300 eggs. Mosquito larvae, commonly called “wigglers” or “wrigglers,” must live in water from 7 to 14 days depending on water temperature. Larvae come to the surface at frequent intervals to obtain oxygen through a breathing tube called a siphon (*Culex*), while others lie parallel to the surface to obtain oxygen (*Anopheles*) (Fig. 35.14). During growth, the larva molts four times. The stages between molts are called instars. At the fourth instar, the larva reaches a length of almost a half inch. When the fourth instar larva molts it becomes a pupa.

Anopheles mosquitoes are the only mosquito which transmits malaria to humans; they also transmit filariasis and encephalitis. ***Aedes*** mosquitoes are painful and persistent biters, attacking during daylight hours (not at night). They do not enter dwellings, and they prefer to bite mammals like humans. *Aedes* mosquitoes are strong fliers and are known to fly many miles from their breeding sources. *Aedes* carry yellow fever, dengue, and encephalitis. ***Culex*** mosquitoes are also painful and persistent biters, but they prefer to attack at dusk and after dark and readily enter dwellings for blood meals. Domestic and wild birds are the preferred targets. *Culex* are generally weak fliers and do not move far from home, although they have been known to fly up to 2 miles. *Culex* live only a few weeks during the warm summer months. Those females which emerge in late summer search for sheltered areas where they “hibernate” until spring. Warm weather brings them out in search of water on which to lay their eggs. *Culex* mosquitoes carry encephalitis, filariasis, and West Nile virus.

Mosquitoes are the most important group of arthropods in terms of disease transmission and, as pests, produce an irritating dermatitis. To be effective vectors for malaria, female *Anopheles* mosquitoes must feed frequently on humans, must be relatively susceptible to the malarial gametocytes, must live long enough for the malaria parasite to complete the life cycle, and must be present in sufficient numbers to maintain transmission of the parasite. Blood feeding follows a circadian rhythm, with most species feeding at night or during the twilight hours, either right after sunset or before sunrise. The biting cycle has control implications, since mosquito nets are effective against nocturnal species, especially those that bite after midnight. Mosquitoes are not limited to ground level elevation, but in forests can be found from the surface up into the canopy where birds and primates live.

In the tropics, breeding usually continues throughout the year. Important species within the United States include *Aedes aegypti* (yellow fever, dengue fever), *Aedes albopictus* (yellow fever, dengue fever, LaCrosse encephalitis virus), *Ochlerotatus* (*Aedes*) *sollicitans* (Eastern equine encephalitis [EEE]), *Aedes vexans* (EEE), *Anopheles quadrimaculatus* (malaria in the eastern United States in the early 20th century), *Culex nigripalpus* (St. Louis encephalitis [SLE]), *Culex quinquefasciatus* (SLE, West Nile virus [WNV]), *Culex salinarius* (SLE, WNV, EEE), and *Culex* *tarsalis* (Western equine encephalitis [WEE], SLE). Mosquito-borne diseases cause millions of deaths worldwide every year, primarily in children and the elderly in developing countries.

Myiasis

Myiasis is the infestation of live human and vertebrate animals with dipterous larvae which feed on the host's dead or living tissue, liquid body substances, or ingested food (Tables 35.4 and 35.5). Maggots can infest any organ or tissue accessible to fly oviposition; most cases probably occur when a female fly lands on a human host and deposits either eggs or larvae. Human myiasis is found worldwide, with more species and greater abundance in poor socioeconomic regions of tropical and subtropical countries. In countries where it is not endemic, myiasis is an important condition, where it can represent the fourth most common travel-associated skin disease.

The order Diptera contains insects that are commonly known as true flies. The presence of a single pair of functional wings with a reduced hind wing, termed halteres, distinguishes true flies from other insects. There are approximately 150,000 species in 10,000 genera and 150 families. This order contains most of the insects transmitting diseases in humans.

Human infestations have been found in the breast, urinary bladder, colon, penis, gums, brain, carcinoma of the scalp, and various skin lesions (20–27). It is also important to realize that nosocomial transmission can occur; myiasis cases have occurred in hospital patients, often those who are debilitated (28–31). Specimens should be killed, preserved in hot (60°C) 70% alcohol, and then submitted to the laboratory for identification. Myiasis-producing members of the Diptera of medical importance can be seen in Tables 35.4 and 35.5.

For business or pleasure, more people than ever before are traveling throughout the world. Since 3 to 10% of these travelers experience skin, hair, or nail disorders related to their travels, it is more and more likely that Western physicians will be expected to treat exotic infections imported from different countries. Tungiasis and furunculoid myiasis are two typical disorders of intertropical regions. These are conditions induced by the presence of arthropod larvae or eggs in the skin. It will become more important for physicians to recognize these and other conditions, many of which they may have not seen previously.

In cases of myiasis, it is important to make the correct identification of the larva. This helps to clarify how the infestation was acquired, supports the treatment plan, and suggests preventive measures. After removal, the maggot should be killed by immersion for 30 s in very hot (enough to produce vapor) but not boiling water, which prevents decay and maintains the natural color. Larvae should then be preserved in a solution of 70% to 95% ethanol. Killing the larvae directly in a preservative tends to putrefy and shrink the specimen(s). Isopropyl alcohol (70%) can be used instead of 70% ethanol. Formalin is not recommended, because it causes excessive hardening of the larval tissues, making processing more difficult.

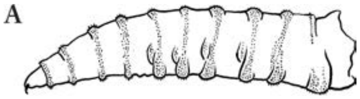
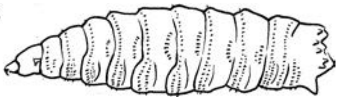
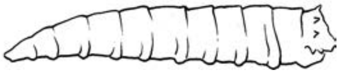
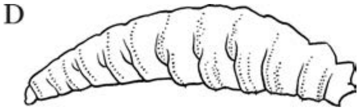
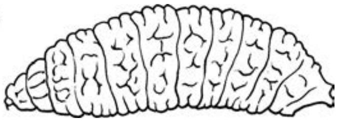
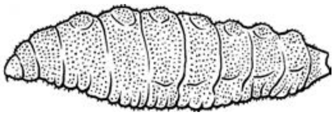
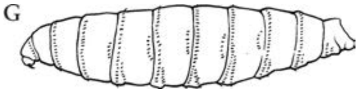
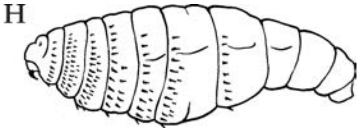
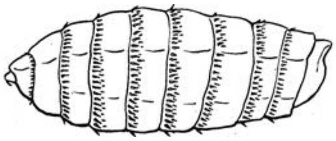
The identification of larvae found in human tissues is based on a number of morphologic characters, including (i) structure of the chitinized mouthparts, (ii) abdominal spiracles, and (iii) structures of the cuticle (these vary depending on the particular instar larva and the number of molts). Occasionally, if living mature larvae are submitted for examination, they can be held until they pupate and hatch; the adult flies can then be examined for identification. Doppler ultrasonography has also been used to evaluate the number of larvae present per lesion; this approach helps avoid misdiagnoses and treatment delays (32). An excellent Internet resource to help identify the species when a trained expert is not present is the Natural History Museum (London, United Kingdom) website (http://www.nhm.ac.uk/research-curation/research/projects/myiasis-larvae/intro-myiasis/index.html) (accessed 6/3/14) using the option, "launch the key to myiasis-causing larvae."

Pseudomyiasis refers to the accidental ingestion of dead or living fly larvae with no associated pathologic changes or symptoms. The term "sanguinivorous myiasis" has been used to refer to superficial attachment of larvae during blood sucking.

Medicolegal Forensic Entomology

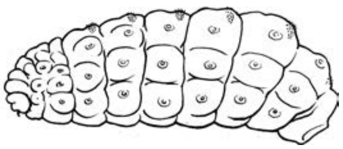
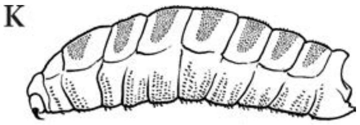
Forensic entomology has become a recognized discipline that is concerned with the application of information on insects and other arthropods to legal issues. The most common applications deal with the determination of death, manner of death, movement of the corpse, and other aspects of the investigation. Insects occurring in carrion can be assigned to four groups. Those that feed on the carrion, including blowflies, arrive within a few hours of death. They are followed by species that feed on the body, species that feed on its inhabitants, and species that use the corpse as an extension of their natural environment. Various stages are identified in the decomposition of the corpse and are important in helping set the time of death: (i) autolytic and microbial decomposition, during which blowflies oviposit; (ii) active decomposition by insects; (iii) advanced decomposition, primarily by coleopteran beetle larvae; (iv) microbial decomposition, which takes over when coleopteran beetle larvae leave and ends in mummification; and (v) separation of the skeleton into individual bones, when the time of death can no longer be determined (20).

TABLE 35.4 Diptera of medical importance in myiasis

Scientific and common names	General habits	Body site(s)[a]	Diagram
Calliphoridae			
Calliphorinae (metallic group)			
Chrysomyia bezziana, Old World screwworm (**A**)	Living tissues	Any body site, eye, erosion of bone	A 
Cochliomyia hominivorax, New World screwworm (**B**)	Living tissues	Nasal, aural sites, brain penetration	B 
Lucilia spp., greenbottles	Dead tissues	Skin, GI tract, GU tract	
Calliphora spp., bluebottles (**C**)	Dead tissues	Skin, GI tract, GU tract, nose	C 
Phormia regina, black blowfly (**D**)	Wounds	Traumatic myiasis	D 
Cynomyopsis cadaverina, blowfly	Wounds	Traumatic myiasis	
Calliphorinae (nonmetallic group)			
Auchmeromyia senegalensis, Congo floor maggot (**E**)	Sucks blood from skin	Does not penetrate, detaches after feeding	E 
Cordylobia anthropophaga, Tumbu fly (**F**)	Subcutaneous boils, sloughing, gangrene	Penetrates unbroken skin, usually feet	F 
Sarcophaginae			
Wohlfahrtia spp., flesh flies	Wounds, normal tissue (head)	Skin, sense organs	
Wohlfahrtia magnifica, Old World flesh fly (**G**)	Most common in humans	Skin, sense organs	G 
Sarcophaga haemorrhoidalis	Dead tissues	GI tract, also skin, GU tract, nose, sinuses	
Sarcophaga carnaria	Living tissues	Vagina	
Cuterebridae			
Dermatobia hominis, human botfly (**H**)	Living tissues	Skin, subcutaneous boils, brain	H 
Cuterebra spp., North American botflies		Skin, eye	
Gasterophilidae			
Gasterophilus spp., horse (stomach) botflies (**I**)	"Larva migrans" cutaneous migration	Skin, migrate to eye	I 

(continued)

TABLE 35.4 Diptera of medical importance in myiasis *(continued)*

Scientific and common names	General habits	Body site(s)[a]	Diagram
Hypodermatidae			
Hypoderma spp., warble flies (**J**)	Skin penetration, extensive larval wandering (limbs)	Skin, eye, nasopharynx, chest, back, limbs, etc.	**J** 
Hypoderma lineatum, lesser cattle warble fly			
Hypoderma tarandi, reindeer warble fly			
Cephenemyia ulrichii, moose throat botfly		Eye	
Oestridae			
Oestrus ovis, sheep nasal botfly (**K**)	Living tissues, resembles conjunctivitis	Nasopharynx, eye, sinuses	**K** 

[a] GI, gastrointestinal; GU, genitourinary.

The entomologist must determine the time the insect attack began; many factors influence the confirmation of this time: cadaver location (whether it was exposed to sun or shade); whether the cadaver was covered, buried, or open to the elements; whether it was wet or dry; and ambient and ground temperatures. Often a pig is used to simulate the corpse conditions and to determine various rates of decomposition and insect activity; this is particularly helpful if conditions in a particular ecoclimate are not known.

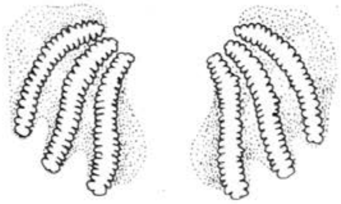
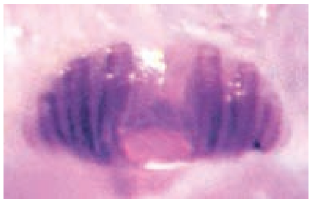
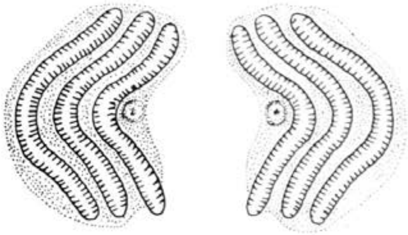
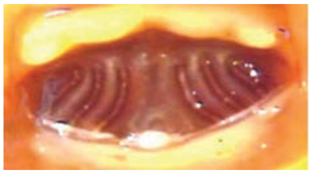
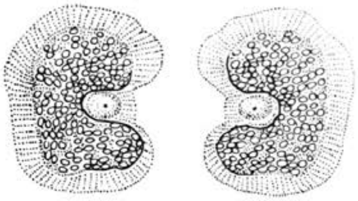
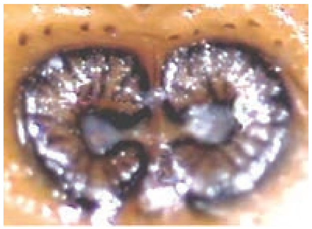
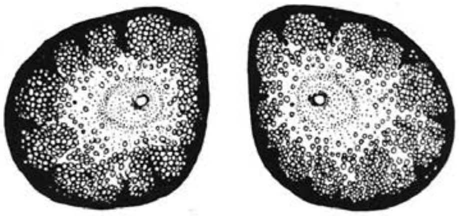
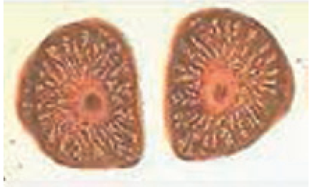
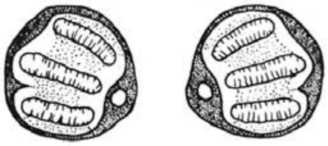
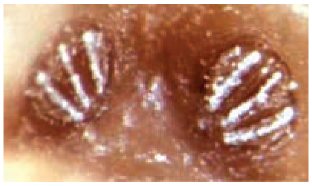
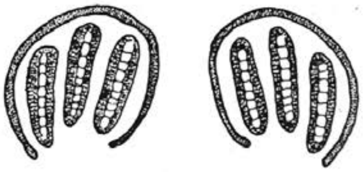
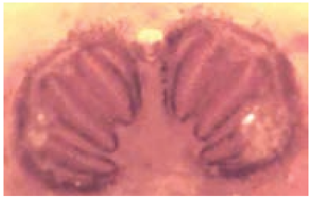
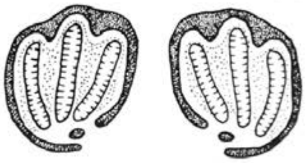
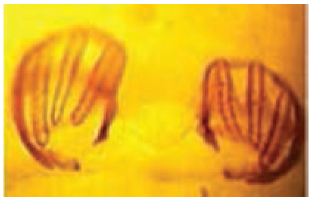
Because the length of the oldest maggots recovered from a body often provides an accurate estimate of the time of death, it is important to ensure adequate fixation without subsequent shrinkage. Studies have shown that maggot shrinkage can lead to underage errors, thus leading to differences of many hours. It has been found that maggots killed in boiling water and then placed in appropriate preservatives do not shrink. The length of the larval crop, which can be useful in age estimates of postfeeding larvae, is not altered significantly when using this approach. Standardized handling and fixation of maggots at the crime scene are critical to valid and consistent interpretations (33).

It is also possible that fly pupae may contaminate forensic entomology samples at death scenes if they originate from animal carcasses or other decomposing organic material rather than from human remains. These contaminants may falsely increase postmortem estimates if no pupae or puparia are actually associated with the body (34).

To permit rapid identification of arthropods, random amplified polymorphic DNA typing has been used to support classic morphologic and forensic analysis of maggots found on a human corpse. This approach has been used to determine if maggots found on the body match those on the outside of the body bag or wrap or if they match the pupae found on the floor or the ground under the corpse. This type of methodology can discriminate between closely related flies and beetles found on corpses (35). This approach has been very helpful in setting the time of death for bodies found less than 1 month after death. After this point, larval insects can be very difficult to identify morphologically when the stages are immature and only fragments of pupae and first-instar larvae are found. An easy and objective method of insect identification is DNA typing; this new approach provides excellent information in a relatively short time (36).

Another relatively new approach involves forensic analysis of DNA recovered from a larva's gut, which can be used to identify the larval food source. This method for associating a maggot with a corpse could be useful, particularly in situations where remains were removed from a suspected crime scene, when an alternative food source is near the scene or the body, or in a chain-of-evidence dispute. However, since maggot gut content analysis represents a new approach, many of the limitations of the technique have not yet been identified. In spite of possible limitations, it appears that third-instar larvae actively feeding on a corpse could be considered an excellent source of human DNA (37, 38).

TABLE 35.5 Species infecting the skin, eyes, nose, and ears in myiasis; examples of spiracles (stigmal plates)[a]

Representative species	Common name	Spiracle (stigmal plates)
Dermatobia hominis	Human botfly	 
Gasterophilus intestinalis	Horse botfly	 
Hypoderma bovis	Cattle botfly	 
Oestrus ovis	Sheep botfly	 
Calliphora sp.	Flesh fly	 
Wohlfahrtia sp.	Flesh fly	 
Sarcophaga sp.	Flesh fly	 

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[a] Adapted from references 20 and 21; illustrations by Sharon Belkin.

Insects can also assist in determining the cause of death and can serve as reliable alternative specimens for toxicologic analyses in the absence of tissues and fluids normally used for such purposes. The presence of drugs and/or toxins in decomposing tissues may alter the rate and patterns of development of arthropods feeding on these tissues. Insects can also accumulate heavy metals and toxic compounds, or their by-products, in their tissues (39).

Studies are under way to assess the utility of microsatellite markers to track the movements and sources of corpses via blowflies (40). Cuticular hydrocarbon analysis on first-instar larvae has been applied to three forensically important blowflies, *Lucilia sericata*, *Calliphora vicina*, and *Calliphora vomitoria*, using gas chromatography-mass spectrometry (GC-MS) and principal component analysis. The results show that each species holds a distinct "fingerprint" hydrocarbon profile, allowing for accurate identification to be established in 1-day old larvae. This GC-MS-based technique could enhance the identification process, not only for forensically important species, but also for other entomological samples which are hard to identify using morphological features (41).

Types of Myiasis Categorized According to Affected Tissues

Cutaneous and Mucocutaneous Tissues (Eye, Nose, and Ear). Larval penetration of cutaneous and mucocutaneous tissues causes various degrees of pathologic abnormalities, ranging from an irritating pruritus to invasion of the eyes, brain, nasopharynx, bones, ear canal, vagina, tongue, and open wounds (Fig. 35.15). The only known therapeutic procedure, other than applying local palliatives, is surgical removal and treatment for secondary bacterial or fungal infections (if these occur). Examples of representative fly larvae and their stigmal plates are seen in Table 35.5. *Dermatobia hominis* and *Cordylobia anthropophaga* are the most common causative agents of furuncular myiasis, while lesions from *Cuterebra* sp. and *Wohlfahrtia* sp. are also seen. The stigmal plates or spiracles (respiratory openings) from mature fly larvae are characteristic for each species and can be valuable in identification. The larvae orient themselves so that these spiracles are normally at the surface of the host skin, etc., to allow oxygen exchange by the larvae. The typical furuncular lesion is a papule or nodule with a central area that exudes serosanguinous or purulent fluid. In the central pore, the posterior part of the larva can be seen; the respiratory spiracles (stigmal plates) can also be seen, which can be confused with tiny black eyes. Although a furuncle-like lesion is the most common presentation, variations have been described, including vesicular, bullous, pustular, erosive, resembling a bruise, and ulcerative lesions. Pruritus, pain, and movement sensation are the most reported symptoms.

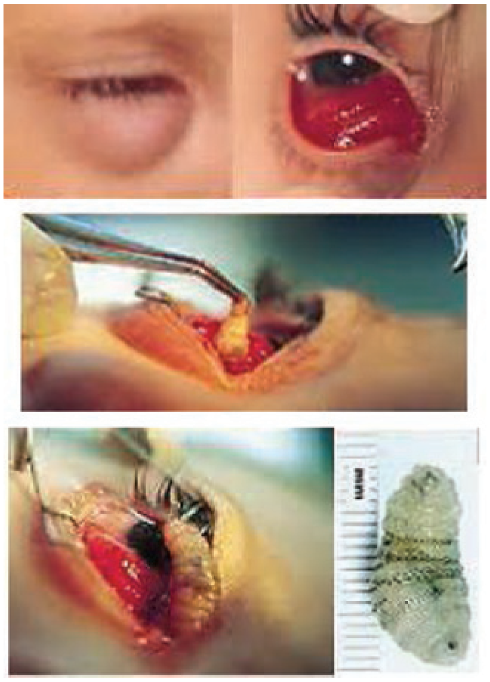


Figure 35.15 Myiasis. (Top) Eye infection with fly larva (*Dermatobia hominis*). (Middle and bottom left) Removal of larva. (Bottom right) *Dermatobia hominis* larva; note the spines (dark bands) (courtesy of reference 42).
doi:10.1128/9781555819002.ch35.f15

***Cuterebra* sp.** is an autochthonous facultative cause of cutaneous and ocular myiasis in humans living in North America. Most cases occur in children, involving the face, scalp, neck, shoulders, or chest. Infestations tend to occur in August, September, and October.

Differential diagnoses of furuncular myiasis are furuncle, insect bite, insect prurigo, pyoderma, inflamed cyst, tungiasis, soft tissue infections, cellulitis, periductal mastitis, benign mass, inflammatory carcinoma, herpes simplex, and delusional infestation. Patient history is critical and may reveal relevant travel history or underlying condition(s). Identification of the larva or larvae can be achieved with appropriate specimen collection and fixation. Also, systemic inflammation, peripheral eosinophilia, and elevated IgE may also be confirmed. Although biopsy and routine histology are often performed, the identification of the particular larval species based on larval fragments alone may be somewhat difficult.

Therapy consists of three general techniques: (i) the application of a toxic substance to the larva and egg, (ii) the production of localized hypoxia to force the emergence of the larva, and (iii) the mechanical or surgical removal of the maggots (27). Petrolatum is often used to occlude the breathing mechanism, while polymyxin B is a sterile option. Occlusion may be required for 24 h or more. It is important not to rupture the maggot, thus causing an inflammatory reaction and possible secondary infection.

Intestinal Tissues. Female flies often deposit eggs or larvae on food that is then eaten by humans. Occasionally, some eggs or larvae survive, become temporarily lodged in the intestinal crypts and folds, and partially or completely develop before being passed in the stool (43). Patients may be asymptomatic, or they may present with larvae in the stools, abdominal pain, nausea, vomiting, anal pruritus, or rectal bleeding. The presence of the maggots may cause an inflammation of the intestinal mucosa. Myiasis agents associated with this type of accidental myiasis are *Fannia canicularis*, *Sarcophaga* spp., *Hermetia illucens*, *Muscina stabulans*, *Megaselia scalaris*, *Eristalis tenax*, *Musca domestica*, *Phormia regina*, *Lucilia cuprina*, and *Stomoxys calcitrans*. Around 50 species of fly larvae have been reported, either positively or questionably, from cases of enteric myiasis. These species are principally members of the families *Muscidae*, *Calliphoridae*, and *Sarcophagidae*, which commonly deposit their eggs or larvae on cold meat, cheese, and other food of humans and are thus ingested; the eggs may be swallowed with contaminated food, pass through the intestine unaffected, and be excreted in feces.

Other Sites. There are reports of larvae recovered from urine, the vagina, and the lungs (accidental inhalation of either a gravid female fly or airborne eggs) (9).

Types of Myiasis Categorized According to Tissue Viability

Specific Myiasis. The larvae attack only living tissues of humans and animals, usually cutaneous and subcutaneous areas but also the nose, mouth, sinuses, eyes, ears, anus, and vagina. There are generally six dipterous families represented in this type of infestation (Table 35.4).

Semispecific Myiasis. The larvae breed on the bodies of dead animals and attach to dermal and subdermal areas and cavities. This type of myiasis is represented by four families of dipterous flies (Table 35.4).

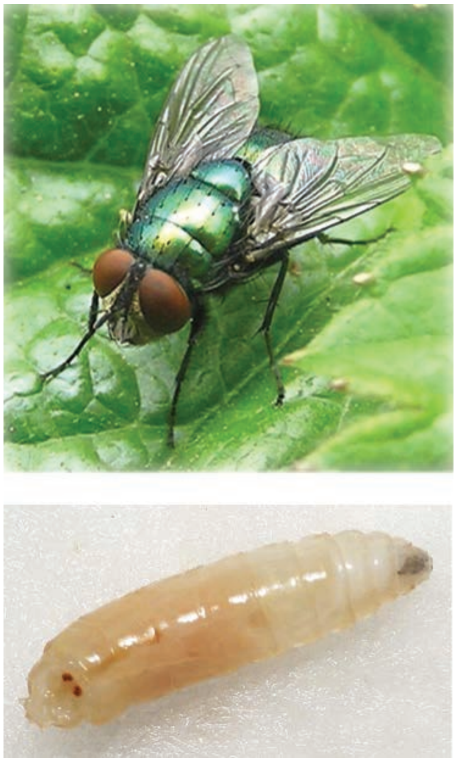
Accidental Myiasis. Infestations are usually accidental and occur infrequently. They are usually in the gastrointestinal tract and are acquired by ingesting food contaminated with larvae. Although the eggs are usually digested or fail to hatch, some may survive, become lodged in the crypts, and develop through part of their life cycle before leaving the body. They may also invade the ears, nose, sinuses, and urinary passages. There are approximately 15 families of flies whose members are implicated in this type of infestation.

Dipterous Flies Involved in Cases of Myiasis

Calliphorid Flies (Blowflies) (*Cochliomyia*, *Phaenicia*, and *Phormia* spp.). Adult blowflies feed on material found in wounds or decaying flesh; females lay their eggs in batches of 200 to 300 in the flesh of dead animals. The eggs usually hatch within 24 h and feed on exudates of wounds or decaying flesh; they penetrate the tissue by using powerful oral hooks, hence the name "screwworm" (Fig. 35.16) (9). Human ophthalmomyiasis has been reported, with one review specifically discussing infection with *Cochliomyia hominivorax* (44). Adult flies have been known to lay eggs on scabs, sores, scratches, pimples, or dried blood; in the healthy mucous membranes of the eyes, nose, mouth, or vagina; and in the umbilicus of neonates. Recent human infestations with *C. hominivorax* include the axilla, nose, palate, and mastoid cells. Blowflies often appear shiny or coppery green to shiny black.

An unusual case of *Phormia regina* myiasis of the scalp was reported in 1988. *P. regina*, the black blowfly, causes

Figure 35.16 *Lucilia* (*Phaenicia*) sp. (blowfly). (Upper) Adult fly (courtesy of Derrick Ditchburn, http://www.dereila.ca/whispers/flies3.html). (Lower) Larva prior to pupation. doi:10.1128/9781555819002.ch35.f16

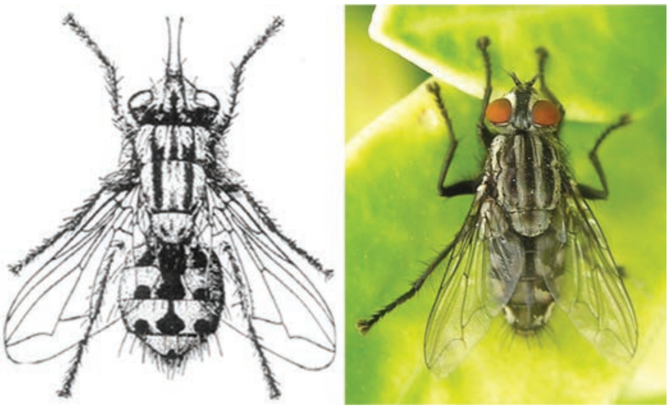


facultative myiasis, usually invading wounds, benign and malignant ulcers, and other diseased tissue; rarely are normal tissues involved. However, in this case, there were no recognizable associated infected wounds or tumor. The patient was a 64-year-old homeless woman who had numerous scratches on her scalp. There is speculation that infection started with the repetitive scratching and that the open suppurative scalp wounds, which were never washed, were likely sites for egg deposition. *P. regina* is very common throughout the United States, especially during April to September (45).

Flesh Flies (*Sarcophaga*, *Wohlfahrtia*, and *Parasarcophaga* spp.). The adult flies feed on feces or decaying meat or fish (9); females deposit 40 to 80 first-stage larvae. The larvae penetrate the tissue, thus causing problems depending on the body site (Fig. 35.17).

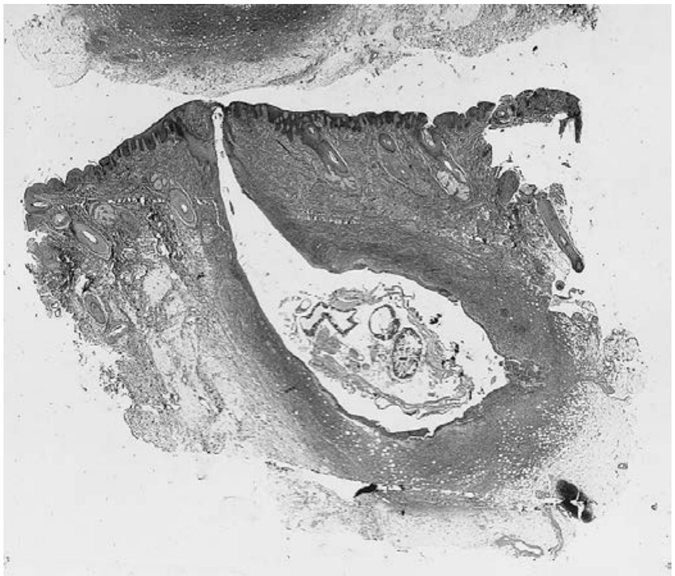
Medically important genera of flesh flies include *Wohlfahrtia* and *Parasarcophaga*. *Wohlfartia magnifica* never develops in carrion, but is found in wounds and natural orifices of warm-blooded animals, including humans. *Wohlfartia vigil* occurs in northern North America, and the larvae are not as invasive, usually being limited to dermal tissues and causing a boil-like lesion. The first reported case of human aural myiasis caused by the flesh fly *Parasarcophaga crassipalpis* was reported in 1987. The patient was a 16-year-old, mentally disabled, nonresponsive Caucasian boy at a public institution in Adelaide, South Australia. Some blood was noticed in the left external ear canal, and 2 weeks later, dark blood was seen discharging from the ear. The physician detected movement in the ear, and that evening a single maggot emerged from the ear. No other maggots were found, and there was no tissue damage in the external ear canal.

Figure 35.17 Flesh fly (*Sarcophagidae*), causes myiasis in humans. (Left, adapted from James MT, U.S. Department of Agriculture Miscellaneous Publication 631, 1947; right, color image courtesy of Derrick Ditchburn, http://www.dereila.ca/whispers/flies3.html.) doi:10.1128/9781555819002.ch35.f17



Botflies (*Dermatobia hominis*, *Gastrophilus* spp., *Hypoderma* spp., *Cuterebra* spp., and *Oestrus ovis*) (Cutaneous, Ophthalmic, Nasal, and Aural Myiasis). The adult female *Dermatobia hominis* fly never feeds but uses food stored up during the larval stage for egg production. Once the eggs are developed, the female fly captures a mosquito or other blood-sucking arthropod and glues the eggs to the abdomen of the arthropod. When the arthropod lands on a warm-blooded host, the young larvae emerge from the egg membranes and, within less than an hour, invade the skin. Lesions commonly occur on the unprotected skin; the tunnel produced is generally perpendicular to the surface and is occupied by a single larva. Eventually, local tissue destruction and accumulation of toxic waste produce throbbing pain and intense itching. The lesion may resemble a bacterial infection with seropurulent fluid (Fig. 35.18–35.21). *D. hominis* is not found within the United States; however, cases are seen in travelers returning from areas of endemic infection that include parts of Mexico and Central and South America.

Figure 35.18 Myiasis. Nodule from the pubis of a 24-year-old woman. She was bitten while in a banana grove in Colombia 2 weeks before this nodule was removed. The fly larva is coiled in a cyst that communicates with the surface. The cyst and tract are lined in part by squamous epithelium. This lesion is characteristic of the "warble" or boil of *Dermatobia hominis*, although the species could not be determined from the section studies. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch35.f18



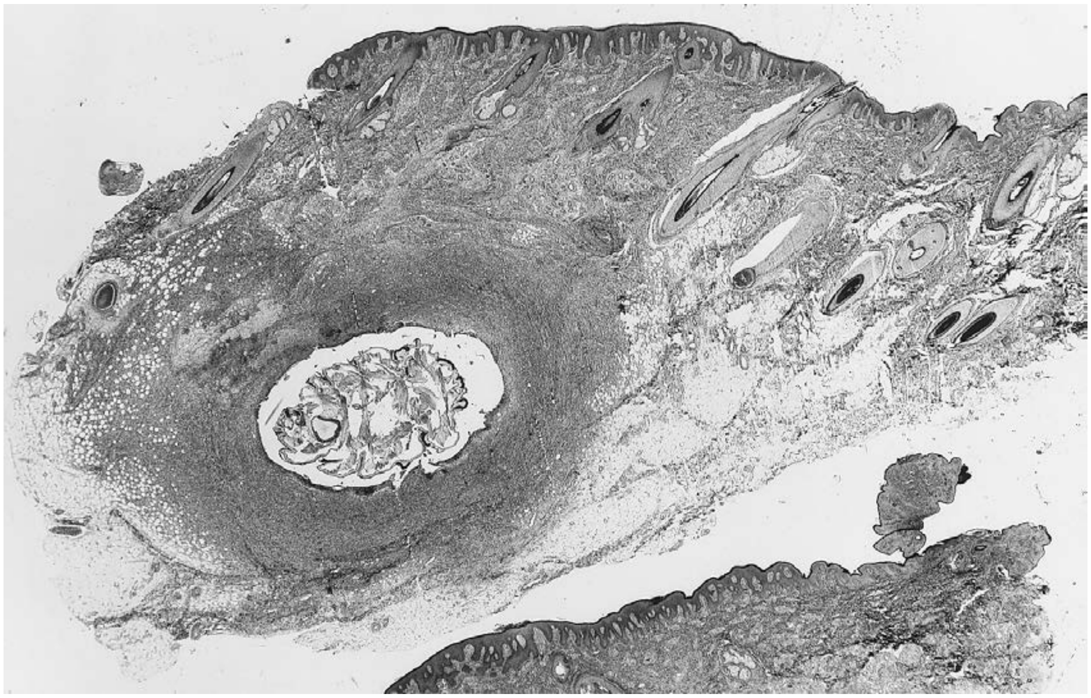


Figure 35.19 Myiasis. Higher magnification of the lesion in Fig. 35.18. The larva is surrounded by an intense inflammatory cell infiltrate composed of neutrophils, lymphocytes, plasma cells, and eosinophils. Magnification, ×9.7. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch35.f19

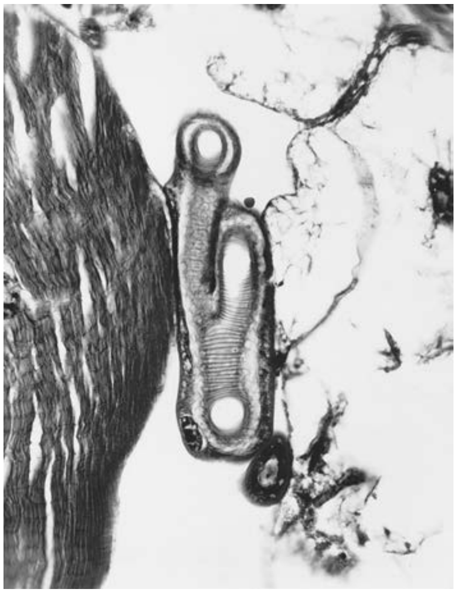


Figure 35.20 Myiasis. A portion of the spiracular system of the larva shown in Fig. 35.18 and 35.19. Note the distinct tracheal rings. Magnification, ×440. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch35.f20

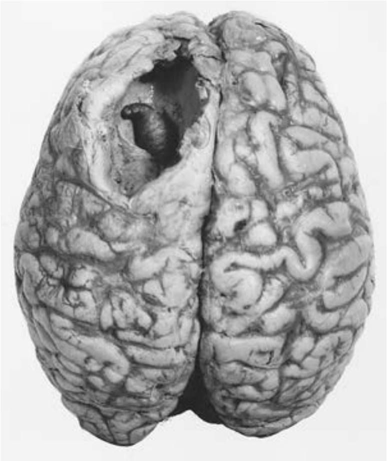


Figure 35.21 Myiasis. Brain of a child in Panama who died of malaria. There were several warbles on the scalp. The larva in the cavity entered through a 4-mm hole that it had bored through the anterior fontanelle. The shape and spines of the larva are consistent with *Dermatobia hominis*. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch35.f21

Although human infestation with *Gastrophilus* larvae is uncommon, the lesion is usually cutaneous. The larvae may advance in the tunnel, with the lesion resembling that of *Ancylostoma braziliense* (cutaneous larva migrans). Ophthalmomyiasis due to *Gastrophilus intestinalis* has also been reported.

Flies of the genus *Hypoderma* most commonly cause dermal myiasis in humans in the United States. The lesions are less serpiginous than those caused by *Gastrophilus* spp., particularly since the larvae bore more deeply into the subcutaneous tissues and produce a more inflamed lesion. However, since the larvae do migrate, they can appear many centimeters from the original site in a month's time. It is important to try to remove the larva before it dies and thus causes a more severe problem due to infection and pus formation.

The genus *Cuterebra* contains more than 20 species of flies native to North America, and the larvae occur as dermal parasites of rodent and rabbit hosts; human cases tend to be somewhat rare. A case of cutaneous myiasis of the eyelid as a result of a *Cuterebra* larva has been reported. The patient was a 28-year-old man with a 3-week history of a swollen, tender nodule on his right upper eyelid. Apparently, 2 weeks before symptoms appeared, he was bitten on his shoulder by an insect. Redness and swelling developed and spread into the neck, face, and right upper eyelid during the following week. The patient was treated for periorbital cellulitis and a secondary *Pseudomonas* infection in the lesion, with no improvement. Two days after the lesion was finally incised, an oval larva emerged and was identified as a second-stage *Cuterebra* larva. Another patient (Fig. 35.22) had a pruritic "mosquito bite" on the chest, which became inflamed and enlarged to approximately 4 cm. A "head" developed, from which the patient expressed this larva. Subsequently, the larva was identified as *Cuterebra* sp.

Figure 35.22 Fly larva removed from a man living in Lynchburg, VA. The patient had a pruritic "mosquito bite" on the chest, which became red and then enlarged to about 4 cm. A "head" developed, from which the patient expressed this larva. The larva was identified as *Cuterebra* sp. Magnification, ×22. (Armed Forces Institute of Pathology photograph.)
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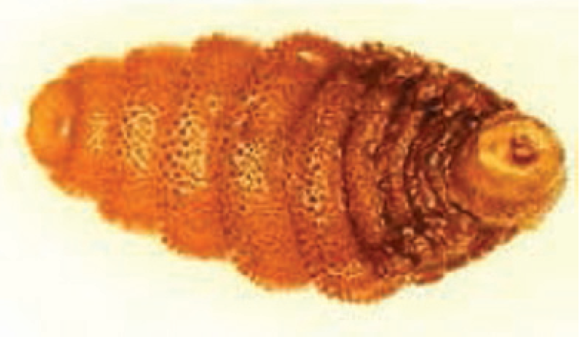


Gravid females of *Oestrus ovis* deposit their first-stage larvae onto the conjunctiva, the outer nares, or the lips or into the buccal cavity. These larvae have large oral hooks and spines that assist their penetration through the mucous membranes. The larvae may burrow into the eyelid, conjunctival sac, or lachrymal duct. Human ophthalmomyiasis is common in the former USSR, northern Africa, and Israel; however, it is rare in Western Europe and the United States. Invasion of the nasal sinuses may result in congestion accompanied by pruritus and frontal headache. Generally, with the exception of the eye, the larvae spontaneously leave the tissues. In cases in which the eye is involved, patients may present with ocular symptoms of foreign-body sensation, irritation, redness, and photophobia. Topical antibiotics and steroid eye drops can be used. Patients may also develop nasal symptoms such as sneezing, nasal discharge, and epistaxis. Larvae can be removed from the conjunctival sac under local anesthetic with no difficulty. Otolaryngology follow-up may demonstrate nasal myiasis as well. Some patients have been treated with ivermectin (46).

Maggot Therapy. Maggot therapy involves induced myiasis performed in a controlled environment by experienced medical personnel. The safety and success of the treatment depend on the selection of an appropriate myiasis species that feeds almost exclusively on necrotic tissue. *Lucilia sericata* is considered the most suitable species for maggot therapy. The larvae are prepared and maintained sterile prior to clinical use (Fig. 35.16).

Maggot therapy has three basic effects on a wound: debridement, disinfection, and enhanced healing. Debridement is the removal of cellular debris and nonviable necrotic tissue from the wound bed. The removal of necrotic tissue, which acts as a microbial substrate, may also reduce the risk of infection. Maggots debride wounds quickly and effectively, without damage to viable tissue. Maggots are photophobic and will naturally move into the deep crevices that may be beyond the reach of a surgeon's scalpel. Reports have confirmed the benefits of maggot debridement therapy in many wound situations (47).

Order Hemiptera (True Bugs)

Bedbugs (*Cimex lectularius*, *Cimex hemipterus*)

Cimex lectularius has a wide distribution, while *C. hemipterus* is primarily tropical. Bedbugs measure about 5 mm long by 3 mm wide, are mahogany brown, and have only vestigial wings (Fig. 35.23). The adult bugs may resemble unengorged ticks or very small cockroaches. Although they

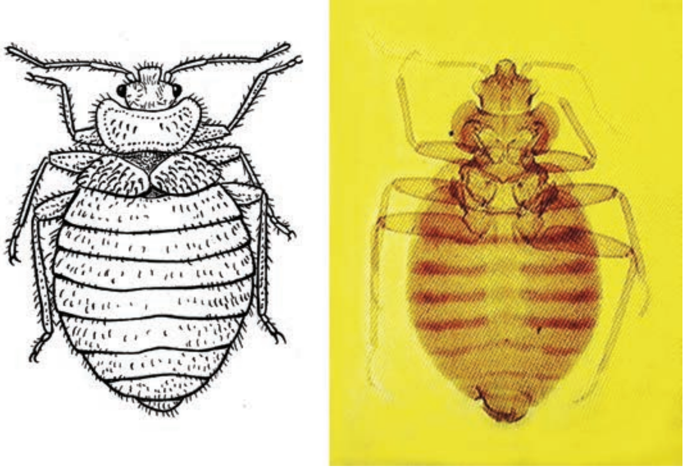


Figure 35.23 Bedbug (*Cimex* sp.). (Left) Adult, 5 mm (illustration by Sharon Belkin, based on an illustration from Najarian HH, *Textbook of Medical Parasitology*, The Williams & Wilkins Co., Baltimore, MD, 1967). (Right) Color image.

doi:10.1128/9781555819002.ch35.f23

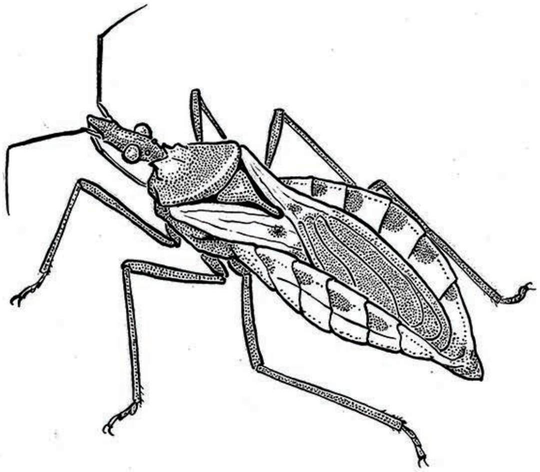
can mechanically transmit pathogenic microorganisms, there is no conclusive evidence that they are natural biological vectors for the transmission of organisms causing diseases. Medical problems associated with these bugs include itching and inflammation caused by their bites. Bedbugs are nocturnal and reach their peak activity before dawn; they tend to hide in cracks during the day. They respond to warmth and carbon dioxide in their search for hosts and will respond to a body that is 2°C or more above ambient temperature. They produce an aggregation pheromone that brings them together. Both sexes of nymphs and adults take blood meals, and blood is the sole food of these bugs. The nocturnal biting of bedbugs can be debilitating to humans, whose sleep is interrupted every night. The presence of the bugs can be suspected by finding their specks of feces and their odor. Bedbugs are very common in developing countries and are becoming more common even in the United States. Travelers may import bugs in their suitcases or belongings, and the bugs then “make themselves at home” in cracks and crevices, behind baseboards, and under mattresses.

Triatomid, Reduviid, Kissing, or Conenose Bugs (Chagas’ Disease)

Triatomid bugs have well-developed wings, are larger than bedbugs, and have a cone-shaped head (Fig. 35.24). These bugs are usually brown or black with orange and black markings on the abdomen where it extends beyond the folded wings. Most species are hematophagous, feeding primarily on mammalian or avian blood. The infective forms of *Trypanosoma cruzi* (Chagas’ disease) are found in the bug’s feces (48). In areas where the infection is more prevalent, the bugs tend to defecate very soon after taking a blood meal. Since the bug’s saliva contains an irritant, the person tends to scratch, thus scratching in the infective forms from the bug feces. The time of the blood meal and the number of feeds during engorgement in relation to defecation are important epidemiologically and are directly related to infection potential.

The earlier a bug defecates during or after feeding, the more likely it is to be an efficient vector. Most triatomid bugs are nocturnal and feed on sleeping inhabitants of the house. They are attracted to the host by warmth, carbon dioxide, and odor. The bites are relatively painless, hence the name “kissing bug.” Obviously it would not be advantageous for the person to wake up before the bugs complete their blood meal and subsequently defecate. However, some individuals have an allergic reaction to the bite approximately 24 to 48 h later; in some cases this is serious. Edema of the eyelids and conjunctivitis may occur (Romaña’s sign) (Fig. 35.25). The edema does not pit on pressure, and the skin is dry. Although Romaña’s sign is thought to be almost pathognomonic for early Chagas’ disease, in an analysis of 300 patients with early disease, unilateral periorbital edema was found in only 48.7%. Currently, it is estimated that 16 million to 18 million people are infected with *T. cruzi* and 90 million to 100 million are at risk. Although most cases occur in Mexico and Central and South America, indigenous cases have been reported from Texas, Tennessee, and California. Serologic evidence places *T. cruzi* as far north as Oklahoma. Five species of kissing bug are found within different parts of the United States: *Triatoma protracta*, *T. gerstaeckeri*, *T. sanguisuga*, *T. rubida*, and *T. rubrofasciata*.

In addition to an irritant, the bug saliva contains an anticoagulant, so that the blood remains unclotted when stored within the bug. This ensures that the blood remains fluid for easier passage into the digestive system of the midgut. In areas of endemic infection, the number of bugs may vary tremendously in houses that harbor the triatomids. When a heavily infested house in Venezuela was torn down, nearly 8,000 triatomid bugs were recovered. It was calculated that in that house the feeding rate was 58 bugs per person per day. The loss of blood per person per month



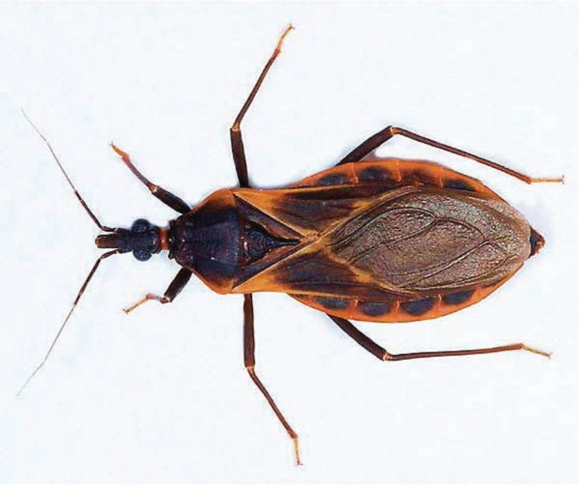


Figure 35.24 Triatomid bug (*Triatoma* sp.). (Upper) Adult bug, 25 mm (illustration by Sharon Belkin). (Lower) Color image of *Triatoma rubida*, the most abundant triatomine species in southern Arizona (courtesy of the CDC Public Health Image Library, photograph by C. Hedgcock). doi:10.1128/9781555819002.ch35.f24

can range from 0.7 to 40 ml; however, in that particular house, it exceeded 100 ml (4).

Most reduviids kill other insects; the name "assassin bugs" is used as a general term. However, bugs associated with the transmission of *T. cruzi* are referred to as kissing bugs. While assassin bugs may bite humans, producing painful lesions, they are not associated with parasite transmission. Often the general names are used interchangeably, thus confusing the issue of actual parasite transmission.

Order Coleoptera (Beetles)

Epicauta. The majority of beetles, representing over 40% of all insects, are not harmful to humans. In some cases, the adult beetles contain vesicating substances such as pederin (paederin) (an amide within body fluids) and cantharidin (a volatile terpene found in the hemolymph) (*Epicauta* spp., *Paederus* spp.). Cantharidin, a bicyclic terpenoid, has an inhibitory effect on protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A). It is stored in the hemolymph, genitalia, and other tissues of the blister beetles, such as those in the genus *Epicauta* (family *Meloidae*) (Fig. 35.26). When these beetles are accidentally crushed against the skin, serious blistering may occur (Fig. 35.26 and 35.27). This type of dermatitis occurs more commonly over the uncovered parts of the body, and the majority of the lesions tend to be on the neck and face. Most of these beetles are seasonal and may be found in large numbers in some fields at certain times of the year. Cantharidin, a natural toxin from blister beetles, used as a medicinal agent since antiquity, has been described to modulate the hepatic leukemia factor competitor NFIL3 and is under clinical evaluation as an antitumor and antimetastatic agent (50).

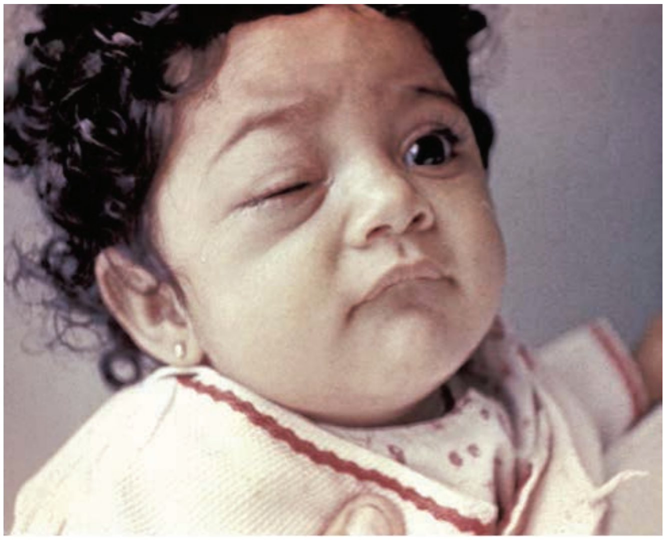


Figure 35.25 Romaña's sign; the assumption is that this is the bite site of the vector, the triatomid bug; unilateral conjunctivitis, palpebral and periorbital edema (courtesy of the Armed Forces Institute of Pathology). doi:10.1128/9781555819002.ch35.f25

Paederus. Beetles in the genus *Paederus* are attracted to the light. They do not bite or sting, but accidental brushing against the beetle or crushing it over the skin provokes the release of its coelomic fluid, which contains pederin, a strong blistering amide that produces a painful blister (Fig. 35.27). There is usually no immediate response to the fluids, but in 24 to 48 h a wheal appears and is followed by blisters. The erythema may last for several months. Ocular and genital involvement is relatively common; it occurs secondary to transfer of the toxic chemical from elsewhere on the skin by fingers. Ocular involvement usually presents with unilateral periorbital dermatitis, or kera-

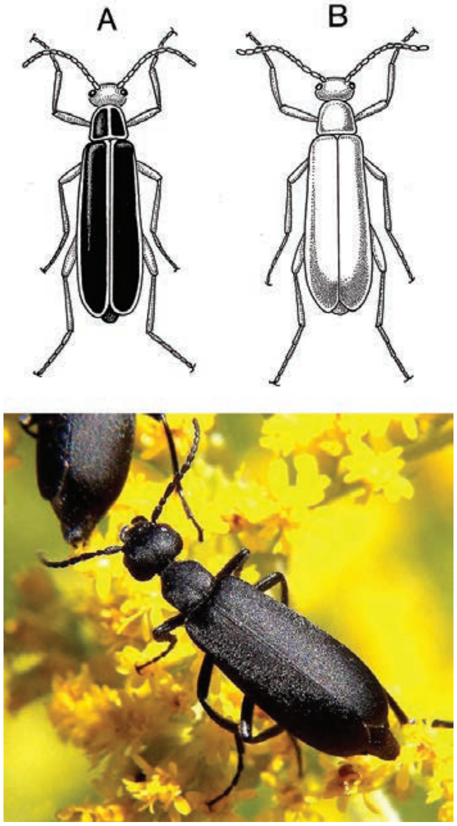


Figure 35.26 Blister beetles. (Upper) (A) Margined (*Epicauta pestifera*); (B) ash gray (*Epicauta fabricii*) (illustration by Sharon Belkin). (Lower) *Epicauta pennsylvanica*, black blister beetle (courtesy of Bruce Marlin, www.cirrusimage.com/beetles.htm). doi:10.1128/9781555819002.ch35.f26

toconjunctivitis, which has been named the Nairobi eye and can cause temporary blindness.

Adult *Paederus* beetles are usually 7 to 10 mm long and 0.5 to 1 mm wide, about one and a half times the size of a mosquito. The head, lower abdomen, and elytra (structure covering the wings) are black and the thorax and upper abdomen are red. It is important to note that *Paederus* beetles are not "blister beetles," which are of the family *Meloidae* (see *Epicauta* above). Blister beetles, which also are widely distributed, release a defensive compound when threatened. The chemical released, cantharidin, a bicyclic terpenoid, is quite different from pederin, an amide.

The manufacture of pederin is largely confined to the female insect. The toxin pederin is the most complex nonproteinaceous insect secretion known and has been used in Chinese medicine for over 1,000 years. Recently, it has been demonstrated that the production of pederin relies on the activities of an endosymbiont (*Pseudomonas* species) within the beetle (51).

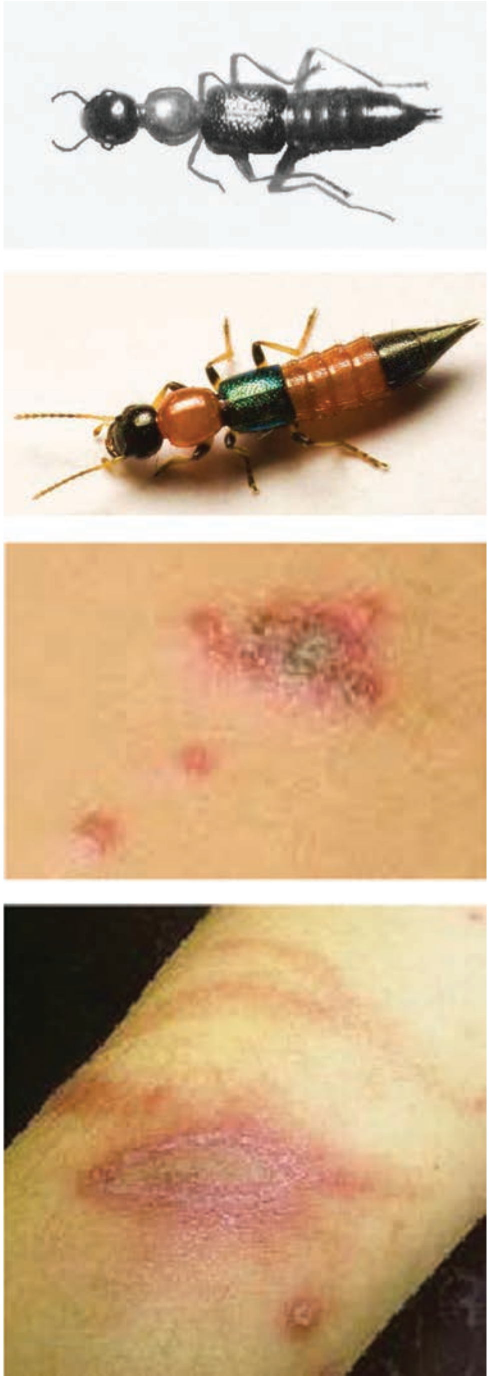


Figure 35.27 (Top) *Paederus laetus*, a beetle of Central America which elaborates a vesiculating toxin. (Row 2) Color image of *Paederus* sp.; magnification, ×4. (Row 3) Contact dermatitis from staphylinid beetle, *Paederus* sp., on the body of a man in Ethiopia. (Bottom) Dermatitis after crushing a beetle on the skin. (Armed Forces Institute of Pathology photographs.) doi:10.1128/9781555819002.ch35.f27

Preventing human/beetle contact is the primary method of avoiding *Paederus* dermatitis. Learning to recognize *Paederus* beetles and avoiding handling or crushing these insects will help decrease these eruptions. If a beetle lands on the skin, it should be blown off or encouraged to walk onto a piece of paper and then removed. The area in contact should be immediately washed with soap and water, and any clothes in contact with the beetle should be washed as well.

***Lytta*.** Another important beetle in the family *Meloidae* is in the genus *Lytta*, which contains the infamous "Spanish fly" from which the well-known aphrodisiac powder is obtained. Cantharidin ("Spanish fly") is a chemical with vesicant properties derived from blister beetles, which have been used for millennia as a sexual stimulant. Its mode of action is by inhibition of phosphodiesterase and protein phosphatase activity and stimulation of beta-receptors, inducing vascular congestion and inflammation. Morbidity from its abuse is significant. The ingestion of live beetles (*Palembus dermestoides*) in Southeast Asia and triatomids in Mexico may have a basis similar to cantharidin (52).

Order Siphonaptera (Fleas) (*Ctenocephalides* spp., *Xenopsylla cheopis*, *Pulex irritans* [Human Flea], *Tunga penetrans*, *Nosopsyllus fasciatus*, *Echidnophaga gallinacea*, and "Sand Fleas")

Fleas are wingless ectoparasites of mammals and birds. While lice are usually host specific, fleas parasitize a number of hosts. It is this ability to move from one host to another that facilitates the transfer of disease from animals, primarily rats, to humans. Fleas are laterally flattened and have no wings. The legs are long and muscular, which allows them to jump (Fig. 35.28). They have piercing-sucking mouthparts, with which both sexes of adults obtain blood meals. They are hosts and transmitters of several pathogenic organisms including the agents of plague and murine typhus and *Dipylidium caninum*, the dog tapeworm. Also, their bite or presence in the skin causes dermatitis or ulcerations.

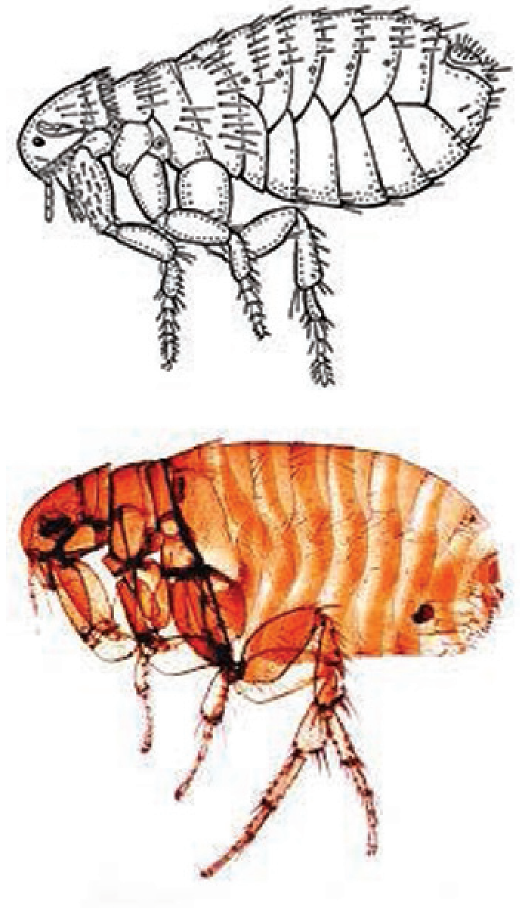


Figure 35.28 Flea. (Upper) Adult flea, 1.5 mm (illustration by Sharon Belkin). (Lower) Color image (from *A Pictorial Presentation of Parasites*: a cooperative collection prepared and/or edited by H. Zaiman). doi:10.1128/9781555819002.ch35.f28

Medically important fleas include *Tunga penetrans* (Fig. 35.29 and 35.30). The adult female *Tunga penetrans* burrows head first into human skin until its head lies within the dermis. The rest of the body is enclosed by the epidermis. A small hole in the keratin remains open, which allows the flea to breathe and discharge feces and eggs to the outside. The penetration of the flea produces no symptoms; however, the expanding flea creates a painful erythematous nodule. Unfortunately, this condition can lead to ulceration, sepsis, tetanus, or gangrene. The fleas can be found on any part of the body, but most often on the feet. Since this flea is a poor jumper, its height range is limited to a few centimeters above the ground. Nodules under a toenail or in pressure-bearing areas of the foot can be extremely painful. The burrowing flea usually appears as a small black spot in the skin. In 95% of cases the injury is in the feet, with a predilection for the plantar region, ankle, and interdigital periungual surfaces. In the remaining 5 to 10% of cases, *T. penetrans* affects other body sites such as hands, elbows, thighs, buttocks, and eyelids. Tungiasis can be misdiagnosed as creeping eruption, dracunculiasis, tick bite, scabies, or myiasis. Flea removal by dissection is the best treatment approach; however, the procedure must be handled carefully. If the flea ruptures, a painful inflammatory reaction may occur, requiring additional intervention. Thiabendazole is usually effective, and niridazole and ivermectin have also been used. Although wearing closed shoes is recommended, this is not always possible, depending on

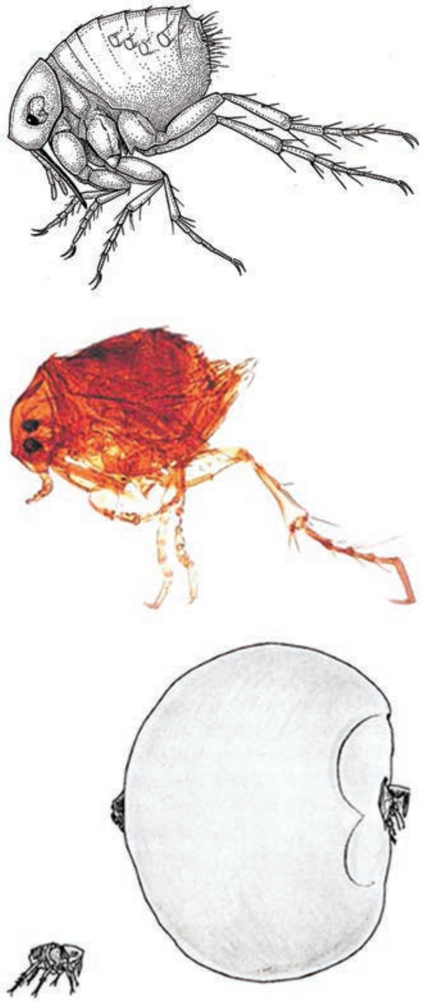


Figure 35.29 Flea (*Tunga penetrans*, chigoe flea). (Top) Adult flea, 1.5 mm (illustration by Sharon Belkin). (Middle) Color image. (Bottom) Diagram of female flea, non-gravid (left) and gravid/full of eggs (right). (Middle and bottom, Armed Forces Institute of Pathology photographs.)
doi:10.1128/9781555819002.ch35.f29

the geographic area. Tungiasis in travelers usually is a benign self-limiting skin disease, whereas inhabitants of areas of endemicity suffer from heavy infestations and severe, frequently debilitating and incapacitating morbidity (53).

Other medically important fleas include *Pulex irritans* (the human flea), *Ctenocephalides canis* (the dog flea), *Ctenocephalides felis* (the cat flea), and *Xenopsylla cheopis* (the Oriental rat flea). Generally the flea bites begin with a wheal around each site within about 5 to 30 min. Itching is common, with the production of an indurated papule within about 12 to 24 h. In people who are sensitive to the bites, a delayed reaction appears in about 12 to 24 h and lasts for a week or longer. The intense itching is often sufficient cause for the patient to consult a physician.

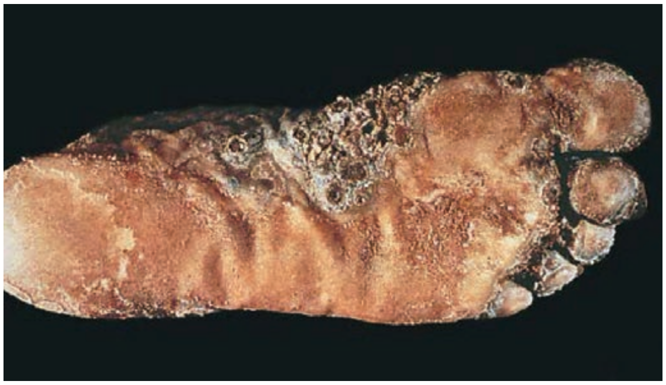


Figure 35.30 Tungiasis. Multiple and irregularly confluent tunga craters on the foot of an African patient. The nonpressure areas (arch and between the toes) are the most severely involved. The weight-bearing portion of the sole tends to be spared. This patient died of tetanus. Note the lesions are healing and crusted. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch35.f30

Cat and dog fleas, *C. felis* and *C. canis*, are major domestic pests in many parts of the world, including North America and Australia; however, the cat flea is most often found within the United States. Both types of flea can feed on either animal. Dogs tend to be more allergic to flea saliva than are cats, but both can have severe reactions to heavy infestation. Cats can remove about 50% of their fleas in a single week, but insecticide dust, spray, shampoo, or other options may be required for removal of all fleas. Flea larvae are very sensitive to drying, and outdoor environments do not generally support survival, which requires a relative humidity of >50%. In the transmission of *D. caninum*, the tapeworm eggs are ingested by the larval stages of the dog, cat, or human flea, where they develop into cysticercoid larvae. When these fleas are then ingested by the definitive host (dogs, cats, or humans), the adult worm develops within 3 to 4 weeks. Most of these infections have been found in children.

Although *Echidnophaga gallinacea* (the sticktight flea) is associated with poultry, it may also feed on humans. Chickens are commonly seen with flea “patches” around the comb, wattles, or even the eyes.

“Sand fleas” is a general term that is used for a number of arthropods, only some of which are actually fleas. In the northern United States, sand fleas are usually dog or cat fleas associated with stray animals. In the western United States, cat or human fleas associated with a number of different animals are called sand fleas. Along the coastal areas, small amphipod crustaceans found in seaweed are also called sand fleas.

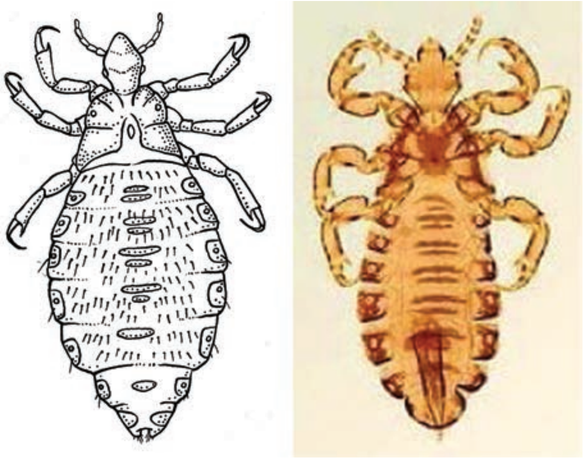
Order Anoplura (Sucking Lice)

The order Anoplura contains the blood-sucking lice (*Pediculus* and *Phthirus* spp.), which have legs with claws adapted for clinging to hairs or fibers. There are three nymphal stages prior to the adult, and all stages feed on blood. In general, the biting lice respond to warmth and smell, avoid high humidity, and are negatively phototactic (i.e., they move toward dark objects). There are several types of human lice: the head louse, the body louse, and the pubic or "crab" louse (Fig. 35.31 and 35.32). The head and pubic lice deposit and cement eggs onto the hair shaft (Fig. 35.33), whereas the body louse deposits eggs on clothing, particularly in the seams. Although they do not specifically transmit any parasites, they do transmit epidemic typhus and louse-borne relapsing fever. Prevention involves both treatment of individuals and mass control, both of which can be difficult in situations such as jails, refugee camps, army barracks, and schools (9).

Body Lice

In a stable population of the body louse, *Pediculus humanus*, more than two-thirds of the lice are present as eggs, about one-fourth are present as nymphs, and only 6 to 7% are present as adults. The adults measure 2 to 3 mm for the male and 2.4 to 3.6 mm for the female. Body lice can cause severe skin irritation; there may be swelling and the development of papules at the bite sites, as well as mild or severe itching. Some individuals may become sensitized to antigens introduced during louse feeding, and they can experience generalized allergic reactions. Removal of skin during itching can also lead to secondary infection. The lice tend to attach to cloth fibers along the seams inside of underwear or other places where clothes touch the body; they tend to prefer wool clothing. *P. humanus* can be removed from clothes by heating the clothes to >60°C for 15 min; this could be accomplished by placing clothes in the clothes drier for about 20 to 30 min.

Figure 35.31 Body louse (*Pediculus* sp.), 1.5 mm (left, illustration by Sharon Belkin; right, courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch35.f31



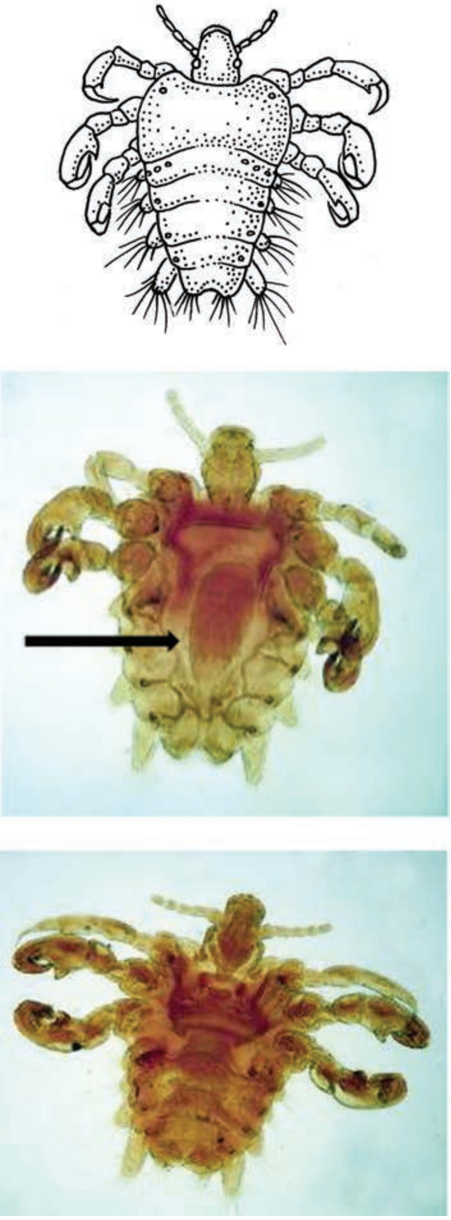


Figure 35.32 Crab louse (*Phthirus* sp.). (Top) Adult, 1 mm (illustration by Sharon Belkin, based on an illustration from Najarian HH, *Textbook of Medical Parasitology*, The Williams & Wilkins Co, Baltimore, MD, 1967). (Middle) Adult female *Phthirus pubis*; note arrow pointing to the egg. (Bottom) Adult male *Phthirus pubis*. (Color images courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch35.f32

Lice infestation dates from approximately 25 million years ago. Clothing lice affect predominantly homeless and

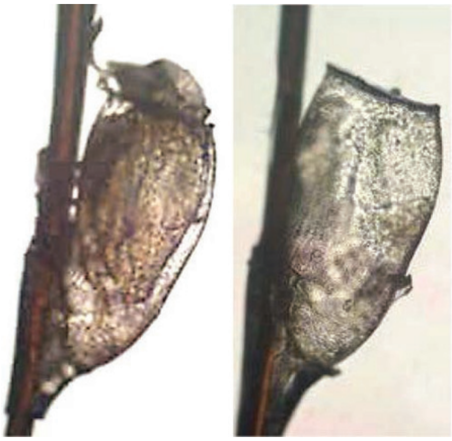


Figure 35.33 Head louse, *Pediculus humanus capitus*. (Left) Egg (nit) on hair shaft with a first-instar nymph starting to hatch out. (Right) Empty shell of the nit from the left image, the nymph having left. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch35.f33

refugee-camp populations and are less prevalent than head lice but far more serious because they transmit at least three deadly bacterial pathogens, those responsible for epidemic typhus (*Rickettsia prowazekii*), trench fever (*Bartonella quintana*), and relapsing fever (*Borrelia recurrentis*). However, head lice have been found to carry *Bartonella quintana* in Nepal, the United States, and Ethiopia (54).

Head Lice

Infestation with *Pediculus humanus capitis* or head lice is a common health problem. It most commonly involves children between 5 and 13 years old and is one of the most common parasitic infections of children. Despite worldwide distribution, this infestation more commonly occurs in crowded living conditions and developing countries. Although most infestations are asymptomatic, pediculosis capitis may result in considerable discomfort, parental anxiety, and embarrassment to the child; unnecessary absence from school and work also has adverse effects on the schoolchildren's academic performance. It is more common in longer hair length, and head-to-head contact is by far the most common route of lice. Head lice may also be transmitted by inanimate objects such as clothes, hats, scarves, combs, towels, bedding, hair brushes, and upholstered furniture or carpets. Hundreds of millions of head louse infestations affect children every year, and this number is on the rise, in part because of increased resistance to insecticides.

Head lice (*P. humanus capitis*) live on the skin among the hairs on the patient's head. Their life span is about 1 month if they are not removed from the body, and the life cycle from egg to adult takes about 3 weeks. Washing the hair reduces the number of nymphs and adult *P. capitis*, and combing with a finely toothed comb removes most of the eggs; combing should be done when the hair is damp. During the washing process, the lice feces are also removed; they can also serve as an irritant during the infestation. It is important to remember that if one person in the family has head lice, the entire family should be treated, since others in the family may be infested but may not be aware of it. Most of the products designed for louse removal are effective, provided that directions are followed. In refugee camp situations where body lice can multiply and spread quickly and easily, DDT dust can be applied to clothing; however, resistance to DDT has arisen, and other insecticides may be required for control.

It is important to remember that hair casts (pseudonits), due to dandruff, can be easily confused with head lice. Examination of the hair shafts with a microscope can help distinguish between the vital nits of an active infestation and hatched empty eggs, as well as actual nit eggs versus pseudonits.

Pubic or Crab Lice

The two distinct species of *Phthirus pubis* occur on separate hosts, the human and the gorilla. Adult pubic lice are dark gray to brown and have a typical "crab" shape. This louse has a smaller body of 1.2 by 0.8 mm, may be lighter in color, and is not as mobile and therefore is harder to see with the naked eye. *P. pubis* occurs on the hair in the pubic and perianal areas of the human body and occasionally the axillae, eyebrows, and beard. The incidence of this infestation has been on the increase, and some think that it may be the most contagious sexually transmitted disease (Fig. 35.32). However, this infection is often found in association with other venereal diseases, including gonorrhea and trichomoniasis.

Transmission can occur by sleeping in the same bed as another infested individual. In patients with an infestation of *P. pubis*, there tend to be blue or slate-gray macules on the skin (maculae caeruleae); these are thought to be either altered patient blood pigments or possibly substances occurring in the salivary glands of the lice. Once these lice have left the host, their survival time is relatively short, with death occurring in less than 48 h at 15°C. If infestation occurs on the eyelids, there may be inflammation of the eyelids. These lice live only on humans, require human blood for survival, and do not infest rooms, carpets, beds, or pets. Because the lice tend to die fairly quickly once they are off the host, it is not necessary to use insecticidal sprays or fogs in the home, work, or school environment.

Phthiriasis palpebrarum is an ectoparasitosis in which *P. pubis* infests the eyelashes. It is rare and it can easily be misdiagnosed as blepharitis. Pediatric phthiriasis palpebrarum can represent child abuse, and the origins of this infection need to be carefully determined. A thorough history can provide information to assess whether further action is needed and, if in doubt, social services should be contacted to ensure child safety. This condition is now grouped under sexually transmitted diseases. It provides a useful tool in forensic medicine in cases of rape and sexual abuse; the host DNA can be identified from the blood meal of the louse via PCR and gene sequencing. This lice infestation is commonly misdiagnosed as bacterial, viral, or allergic conjunctivitis or seborrheic dermatitis. Proper history taking and careful examination are a must for correct diagnosis and appropriate treatment.

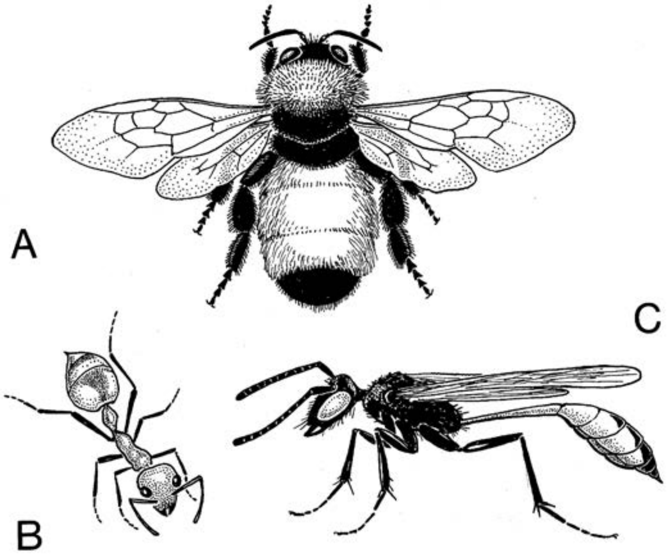
Order Mallophaga (Biting and Chewing Lice)

Biting lice are morphologically similar to other lice and are not very important in human medicine, although they serve as the intermediate host for the dog tapeworm, *Dipylidium caninum*. Infestations are more common in puppies, very old dogs, or those that are sick or debilitated. None of these lice live on humans but are primarily ectoparasites of birds and mammals, feeding on feathers, hairs, and epidermal scales (9).

Order Hymenoptera (Bees, Wasps, and Ants)

The order Hymenoptera contains insects with two pairs of membranous wings, reduced veins in the wings, and hind wings smaller than the fore pair and interlocked with the latter by means of hooklets (Fig. 35.34). There are more than 100,000 described species. Many members of species within this order live together in great structured societies, and reproduction is performed either by many of the female members or, in some cases, by a single individual known as the queen. Members of the group or colony tend to attack intruders.

Figure 35.34 (A) Bee; (B) fire ant; (C) wasp. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.ch35.f34



Bees

Although stings of members of this order normally cause only temporary pain and swelling, anaphylaxis may occur in patients who are sensitized. Within the United States, approximately 50 to 100 individuals die each year from severe anaphylactic reactions to bee and wasp stings (55). Honeybees are not necessarily aggressive, but they tend to be everywhere outdoors during the summer months. During the stinging process, pheromones are released that cause other bees in the vicinity to attack the victim. When a person has been stung, the stinger should be removed, the site should be disinfected, and ice packs should be applied to slow the spread of venom. The stinger can be removed by scraping with a knife or fingernail to prevent further venom from being injected. If the person experiences a severe local reaction, oral antihistamines and topical application of corticosteroids can be used. Systemic allergic reactions can be a life-threatening emergency requiring immediate medical help.

In 1956, Africanized bees began to spread to the American continent from southern Brazil, where original African bees mated with European bees. In 1990, these Africanized bees reached the United States and were found in Texas. They are now found in other states and will probably reach the Canadian border in the future. The spread of the Africanized honeybee to Brazil and then to other areas of South and Central America and the southern United States has been accompanied by extensive publicity. These bees can be easily irritated and provoked to protect the hive, resulting in hundreds of stings, severe reactions, and death. Although the public has received information regarding these bees, the “killer” bees are difficult to differentiate from normal honeybees, particularly visually.

In comparison to the European honeybee, the Africanized bees tend to attack more readily, remain angry for extended periods, and display massive group stinging behavior in defense of the colony and territory. Although their stings are no more toxic than those of domestic bees, death can result from the cumulative effects of numerous stings, numbering from 400 to 1,000 stings, or from anaphylactic shock. It is very likely that we will continue to see more problems and possible deaths due to the Africanized honeybees as their geographic area continues to expand within the United States. As an example, in Venezuela in 1978, before the Africanized honeybees arrived, there were 12 deaths attributed to bee stings; however, after 1978, there were 100 deaths per year. Stings can be treated in the same way; however, in the case of multiple stings, excessive histamine release may result from venom action and not necessarily from an allergic reaction. *Physicians need to be aware of this fact and the possible need to treat for histamine overdose* (56). Victims of these bee attacks usually develop a severe envenomation syndrome characterized by the release of a large amount of cytokines (interleukins [IL] IL-1, IL-6, IL-8) and tumor necrosis factor. Subsequently, such cytokines produce an acute inflammatory response that triggers adverse effects on skeletal muscles, bone marrow, hepatic and renal functions, and cardiovascular, central nervous, and immune systems.

Bumblebees also sting when disturbed; however, they are neither as aggressive nor as prevalent as honeybees. Treatment for stings is the same as for honeybee stings.

Fire Ants

Ants can bite as well as sting; however, most do little harm. One exception is the fire ant, *Solenopsis invicta*, which is endemic in the southeastern United States. The ants measure about 4 to 6 mm long and are reddish brown (red form) or black (black form). Although they look like ordinary ants, their head is a lighter color than the rest of the body. The presence of fire ants can usually be recognized by seeing their characteristic mounds, which are earthen mounds with a diameter of 24 in. and a height of 18 to 24 in. surrounded by relatively undisturbed vegetation (Fig. 35.34, 35.35, and 35.36) (56). The mounds have a freshly tilled appearance. When even slightly disturbed, the ants tend to boil out of their mounds. The workers attach to the skin by biting with their mandibles and then lowering their abdomen to inject the stinger; thus, the victim receives both a bite and a sting. The symptom of a sting has been described as a "burning itch." Within about 24 h, a pustule develops and may remain for about a week (Fig. 35.6). Although some individuals have sustained thousands of stings with no toxic or allergic reactions, some people with hypersensitivity to fire ant venom may have severe reactions from just a few stings. At least 250 million acres within the United States are infested, now reaching as far west as New Mexico, Arizona, and California. In some heavily infested areas, 50 to 400 or more mature colonies may be found per acre. There are actually two species, *S. invicta*, the red form imported from Brazil, and *S. richteri*, the black form imported from Uruguay. The geographic range for *S. richteri* in the United States is much more limited, occurring in northeastern Mississippi, northwestern Alabama, and part of Tennessee (6).

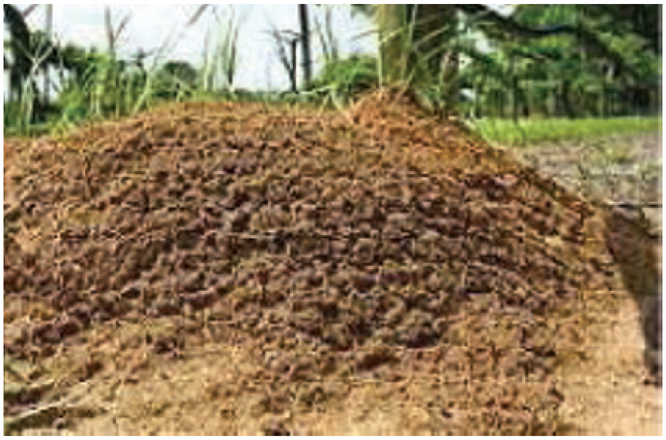


Figure 35.36 Typical mound of the fire ant; the height can reach 2 ft. Often the mound looks like the dirt has just been tilled. (Courtesy of the U.S. Department of Agriculture.) doi:10.1128/9781555819002.ch35.f36

Harvester Ants

Harvester ants, *Pogonomyrmex* spp., are ant species that sting both animals and humans. These ants are red to dark brown and are two or three times as large as fire ants. Harvester ant mounds are usually flat or slightly elevated, measure about 3 to 9 ft in diameter, and are surrounded by no vegetation (Fig. 35.37). There is only one harvester ant species east of the Mississippi River, and it is found

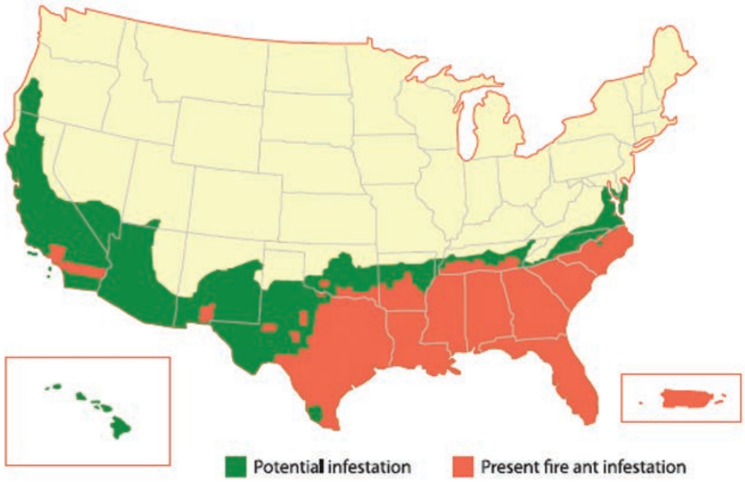


Figure 35.35 Present and potential ranges of imported fire ant infestation (courtesy of the CDC Public Health Image Library, U.S. Department of Agriculture). doi:10.1128/9781555819002.ch35.f35

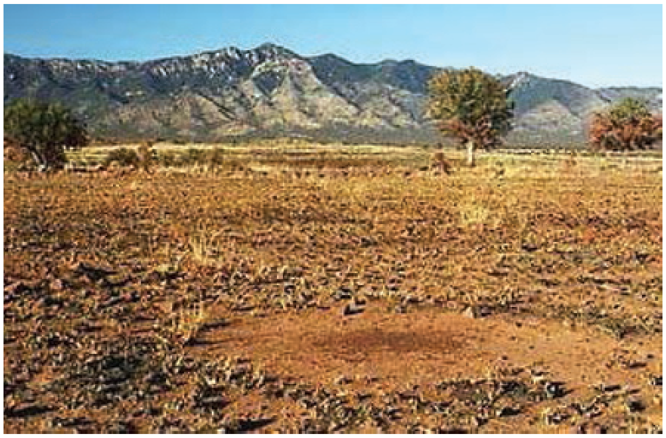


Figure 35.37 Typical flat mound of the harvester ant; the flat area with no vegetation can range from 3 to 9 ft. (Courtesy of the U.S. Department of Agriculture.)
doi:10.1128/9781555819002.ch35.f37

throughout the Southeast. In the western United States, about 20 species occur; three of the most serious are the red harvester ant (*P. barbatus*), the California harvester ant (*P. californicus*), and the Western harvester ant (*P. occidentalis*). The reaction to harvester ant stings is not always localized but may spread along the lymphatics. This causes severe pain in the lymph nodes in the axilla or groin some time after the pain from the original sting has gone. Systemic allergic reactions tend to be uncommon.

Order Blattaria (Cockroaches)

Cockroaches are among the most ubiquitous pests throughout the world. A number of the 3,500 species have become adapted to living with humans and are referred to as "domestic" species that will breed anywhere. They are very active, nocturnal insects; anyone who has turned on the lights in the kitchen late at night remembers the surprise at seeing all the visitors! Cockroaches are flattened dorsoventrally and have long, multisegmented antennae, biting mouthparts, two pairs of wings, two cerci, and legs adapted for running (55). They feed on human food, excreta, sputa, and even bookbindings, paper, and leather.

Mechanical Transmission of Organisms. Although certain flies and dung beetles have been implicated as mechanical vectors of *Cryptosporidium* oocysts, there is every reason to suspect that cockroaches could also do the same (5). When cockroaches feed on infected material, their mouthparts and legs become contaminated; they can then introduce these contaminants into human surroundings. Also, organisms can pass through the cockroach gut and remain fully viable. Some investigators have speculated that the cockroach may be a more significant vector of human pathogens than houseflies, transmitting viruses, bacteria, and parasites to human hosts. In one study of 234 cockroaches trapped from different sites (toilets, parlors, kitchens, and bedrooms) in houses with pit latrines and water systems, bacterial, fungal, and parasitic isolates were identical irrespective of the site and included *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis*, *Citrobacter freundii*, *Enterobacter cloacae*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *S. faecalis*, *S. epidermidis*, *Aeromonas* spp., *Candida* spp., *Rhizopus* spp., *Aspergillus* spp., and *Mucor* spp.; cysts of *Entamoeba histolytica* and of *Balantidium coli*; oocysts of *Cryptosporidium* spp., *Cyclospora cayetanensis*, and *Cystoisospora belli*; ova of *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Enterobius vermicularis*, and *Trichuris trichiura*; and larvae of *Strongyloides stercoralis* (5). Some humans also become allergic to cockroach allergens. Control involves good sanitation, prevention of new entry, and a combination of various pesticides including bait, strips, sprays, and dusts.

Several important species include *Blatella germanica* (German cockroach), *Blatta orientalis* (Oriental cockroach), *Supella longipalpa* (brown-banded cockroach), and *Periplaneta americana* (American cockroach) (Fig. 35.38). These different cockroaches prefer different habitats, with some preferring warm, moist conditions (German cockroach) and others preferring cooler locations (Oriental cockroach). The most common cockroach throughout the United States is the German cockroach, which can be found infesting most buildings. Adult German and brown-banded cockroaches are approximately 15 mm long, while the American and Oriental cockroaches measure 30 to 50 mm long.

Cockroach Allergens. In addition to the mechanical transmission of many types of bacteria, fungi, and parasites, the excrement and cast skins of cockroaches contain a number of antigens that cause allergic responses. Urban allergens include the cockroach, mouse, and rat. Cockroach allergy is common among inner city children with asthma, and exposure to cockroach allergen is associated with more severe disease. Cockroach allergens are one of the major etiologic risk factors for IgE-mediated allergic respiratory illness throughout the world. A high prevalence of cockroach hypersensitivity in atopic (20 to 55%) and asthmatic (49 to 60%) populations has been documented (5). In a survey of day care facilities in North Carolina, detectable levels of indoor allergens were commonly found, including cockroach allergens. For many young children and day care staff, day care facilities might be a source of clinically relevant exposures to indoor allergens. Cockroach allergen avoidance begins with effective pest control, followed by thorough and repeated cleaning. At least 1 to

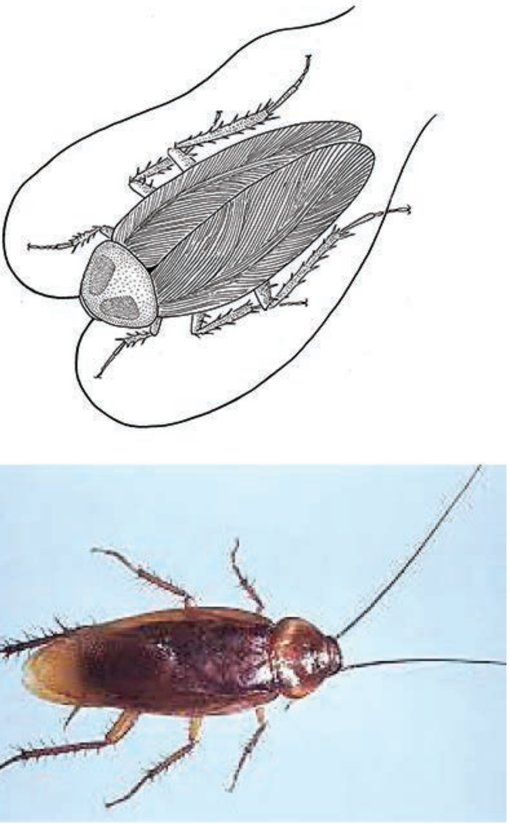


Figure 35.38 American cockroach (*Periplaneta americana*). (Upper, illustration by Sharon Belkin; lower, courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch35.f38

2 months is required to eliminate cockroaches, and an additional 4 to 6 months is required to remove residual allergen. Once allergen levels have been reduced, continued efforts are necessary to maintain the home free of allergen sources. The school environment can also be an important site of exposure to cat, dog, dust mite, and cockroach allergens, which have been detected in the settled dust of many schools. In industrialized nations, the school environment has a lower potential for exposure than the home environment, but schools are significant for allergic individuals whose home environment has been addressed to the extent possible. From a public health perspective, the school environment should be considered a target for primary and secondary prevention.

Class Arachnida (Ticks, Mites, Spiders, and Scorpions)

Subclass Acari (Ticks, Mites, and Chiggers)

The subclass Acari contains the soft and hard ticks, mites, and chiggers.

Soft and Hard Ticks

Although there are over 800 species of ticks throughout the world, only two families of ticks, *Ixodidae* (hard ticks) and *Argasidae* (soft ticks), are known to transmit diseases or illness to humans. The soft and hard ticks are ectoparasites adapted to blood-sucking (mammals, birds, and reptiles) (57–59). There is no hard dorsal plate in the soft tick, and the mouth parts are ventral to the anterior extremity (Fig. 35.39; Tables 35.6, 35.7, and 35.8). In the hard tick, the dorsal plate or shield covers the entire surface of the male but only the anterior portion of the female (Fig. 35.40 and 35.41). The dorsal surface of a tick's body often has color patterns, furrows, or sculpting that allows species determination.

Tick Paralysis. Ticks are responsible for the transmission of viral, bacterial, rickettsial, and protozoan (*Babesia* spp.) pathogens. The salivary secretions of some ticks may produce systemic toxemia (tick paralysis) that can cause death. Paralysis can occur from a tick attached to any part of the body, although the symptoms may be more serious when the tick injects toxin at the back of the neck or along the spinal column. Symptoms may include elevated temperature, rapid ascending flaccid paralysis, and difficulty in breathing and swallowing, and death may result. The onset may be very sudden, within 24 h, or may not appear for several days. The most likely tick involved is *Dermacentor andersoni*, the Rocky Mountain wood tick. Human cases tend to occur in the spring and early summer, when ticks are most active and are seeking a blood meal. Tick paralysis can be particularly dangerous for children; after playing in underbrush or bushes, the child should have all clothing removed and should be carefully examined for the presence of ticks, particularly around the neck and shoulder area (under the hair). The most likely geographic area is in western Montana, Idaho, and Oregon, although cases have been reported from other states including Georgia, Tennessee, Louisiana, and the Atlantic seaboard. Ticks in the genera *Amblyomma* and *Dermacentor* have been involved in cases outside of the western United States, while in other parts of the world *Ixodes*, *Rhipicephalus*, *Hyalomma*, and *Ornithodoros* have been implicated in tick paralysis.

Soft Ticks. The soft ticks have an oval or pear-shaped outline with the anterior body region broadly rounded. The mouthparts are difficult to see from a dorsal view. The soft ticks are inornate and have a granulated leathery appearance. They lack a scutum; however, most species have a centrally located dorsal plate which is covered by tiny mammillae, ridge structures that protrude above the cuticle surface. Subcircular depressions termed discs, representing sites of muscle attachment, occur in characteristic patterns over the dorsal and ventral surface (Fig. 35.39).

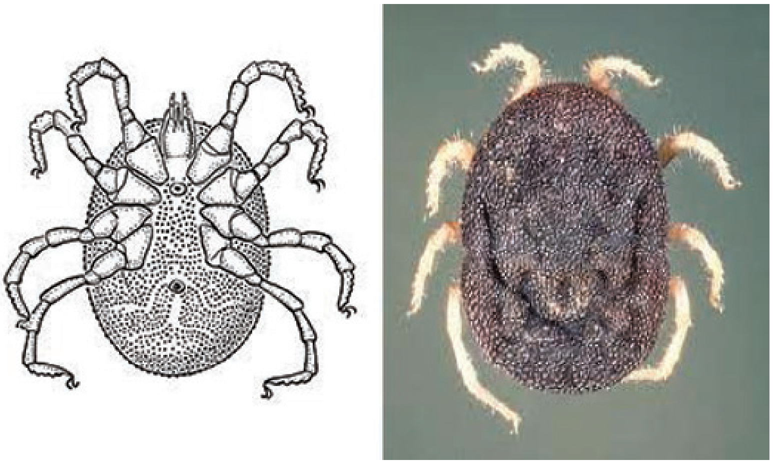


Figure 35.39 (Left) Soft tick, ~12 mm (illustration by Sharon Belkin, based on an illustration from Najarian HH, *Textbook of Medical Parasitology*, The Williams & Wilkins Co, Baltimore, MD, 1967). (Right) Soft tick; note the mouth parts can normally be seen only from the ventral side (courtesy of the CDC Public Health Image Library). doi:10.1128/9781555819002.ch35.f39

Soft ticks feed for short periods of time on their hosts, very similar to that of fleas or bedbugs. The cuticle of soft ticks stretches but does not grow to accommodate the large volume of blood ingested, which may be anywhere from 5 to 10 times their unfed body weight. Soft ticks are considered to be more dangerous than hard ticks because they can transmit disease within minutes of a bite. Differentiation between the soft ticks and hard ticks is relatively easy once an engorged hard tick is recognized as such.

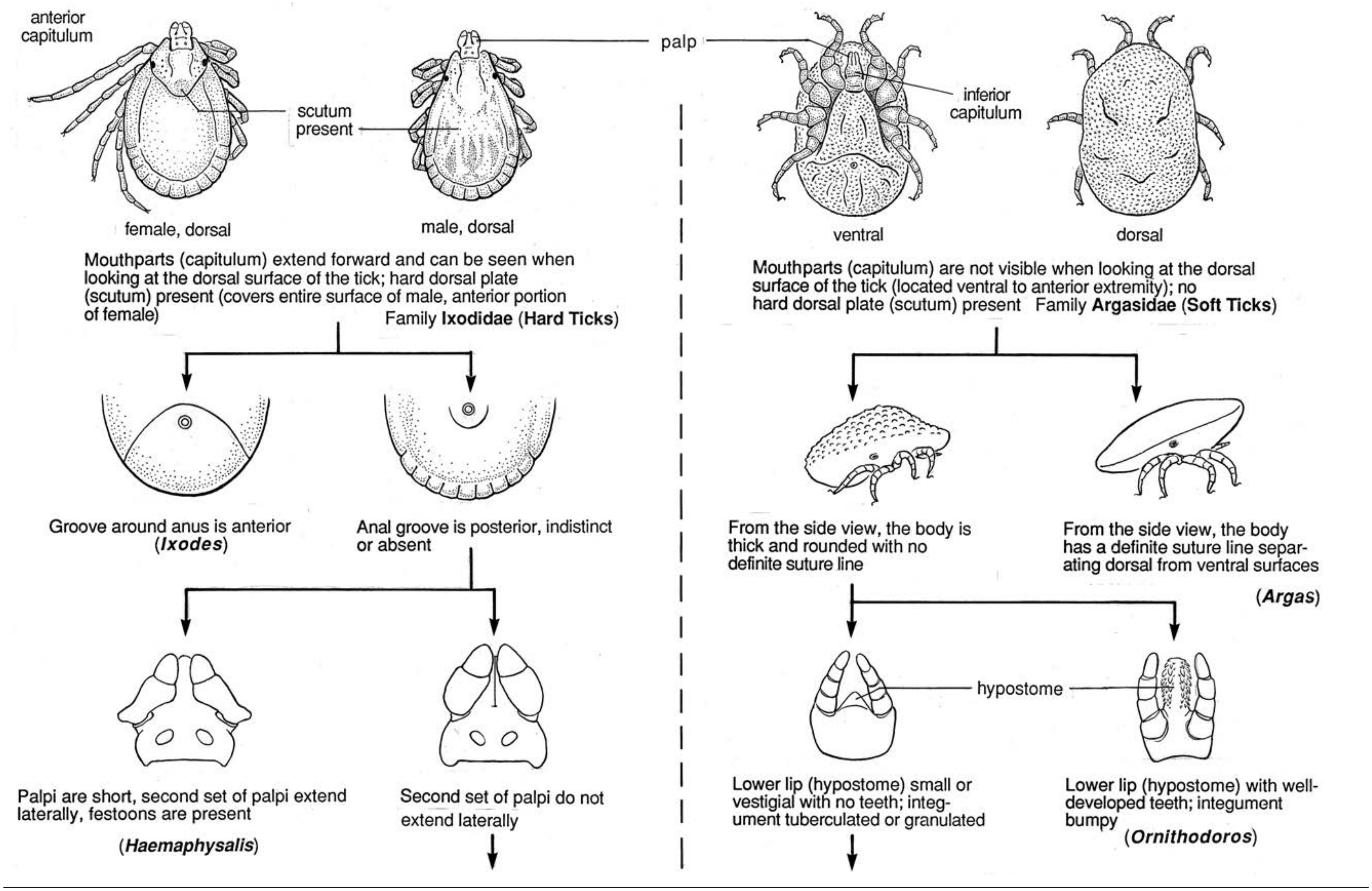
Hard Ticks. Hard ticks are found in habitats that support large numbers of vertebrate hosts such as small mammals, especially mice, ground-dwelling birds, and lizards. Tick larvae tend to remain on the ground, where they encounter potential hosts, but larvae of some species can feed on humans. Nymphs remain on the ground or climb grassy vegetation from which they are able to grasp a passing host, including small to medium-sized mammals, but nymphs of a few species are attracted to larger hosts, including pets and humans. Adult ticks may remain on the ground, but more commonly climb up vegetation, from which they grasp a passing host. They often can be seen at the tops of grass blades or on lower leaves of bushes engaging in "questing." Questing is a behavior in which the tick reaches upward with waving front legs ahead of an approaching host. Adult ticks tend to feed on larger mammalian hosts such as deer, livestock, dogs, and humans.

Ticks feed by making a cut in the host epidermis, inserting the hypostome into the cut, and attaching to the host. Blood is kept flowing through the use of an anticoagulant from the salivary glands of the tick. Some hard ticks form a cement cone around the mouthparts and bite site. Female hard ticks undergo a growth feeding phase characterized by slow continuous blood intake, followed by a rapid engorgement phase occurring during the last 24 h of attachment. Hard ticks have the ability to expand considerably, often resembling a soft tick. Many hard ticks attach themselves to grasses or weeds, waiting for a host to come along; they are also attracted to carbon dioxide. Hard ticks are generally found in brushy, wooded, or weedy areas containing deer, cattle, dogs, small mammals, and other hosts. Hard ticks have to conserve moisture, and so their activity and survival are influenced by temperature as well as humidity. Transmission of a pathogen typically does not occur until an infected tick has attached and fed for at least 24 hours, and transmission of some pathogens does not begin until an infected tick has fed for 48 hours or more.

Identification to the species level among the hard ticks can be difficult. Currently there is a great deal of interest in identifying the ticks that transmit Lyme disease. In the northeastern United States, the primary vector for Lyme disease caused by *Borrelia burgdorferi* is *Ixodes scapularis*, the black-legged tick (formerly known as *Ixodes dammini*, although it now appears that they are a single species) (60, 61). The life cycle takes 2 to 4 years. Larvae are active from August to October, when they are feeding on *Peromyscus leucopus*, the white-footed mouse. The infection rate in mice is approximately 25% and occurs from May to June. Human cases tend to peak in July. A tick needs to feed for 48 h for successful transmission of *B. burgdorferi*; although 50 to 100% of the adult *I. scapularis* ticks are infected, most are removed from the human host prior to 48 h. *I. scapularis* is found in dense brush on islands off the northeast coast and in heavily forested areas inland. In Wisconsin, these ticks occur in areas between the northern coniferous-hardwood and southern deciduous forests.

In western North America, the primary vector of *B. burgdorferi* is *Ixodes pacificus*, the western black-legged tick. These ticks range from sea level to >6,000 ft and are found from California to British Columbia and east to Nevada, Utah, and Idaho. Larvae and nymphs are active from March to June, and adults are found on humans from November to May. The infection rate of *I. pacificus* is

TABLE 35.6 Identification of the more common hard and soft ticks. (Illustration by Sharon Belkin; adapted from National Communicable Disease Center Pictorial Keys, 1969.)



Mouthparts (capitulum) extend forward and can be seen when looking at the dorsal surface of the tick; hard dorsal plate (scutum) present (covers entire surface of male, anterior portion of female) Family **Ixodidae** (**Hard Ticks**)

Groove around anus is anterior (***Ixodes***)

Anal groove is posterior, indistinct or absent

Palpi are short, second set of palpi extend laterally, festoons are present (***Haemaphysalis***)

Second set of palpi do not extend laterally

Mouthparts (capitulum) are not visible when looking at the dorsal surface of the tick (located ventral to anterior extremity); no hard dorsal plate (scutum) present Family **Argasidae** (**Soft Ticks**)

From the side view, the body is thick and rounded with no definite suture line

From the side view, the body has a definite suture line separating dorsal from ventral surfaces (***Argas***)

Lower lip (hypostome) small or vestigial with no teeth; integument tuberculated or granulated

Lower lip (hypostome) with well-developed teeth; integument bumpy (***Ornithodoros***)

(continued)

TABLE 35.6 Identification of the more common hard and soft ticks. (Illustration by Sharon Belkin; adapted from National Communicable Disease Center Pictorial Keys, 1969.) *(continued)*

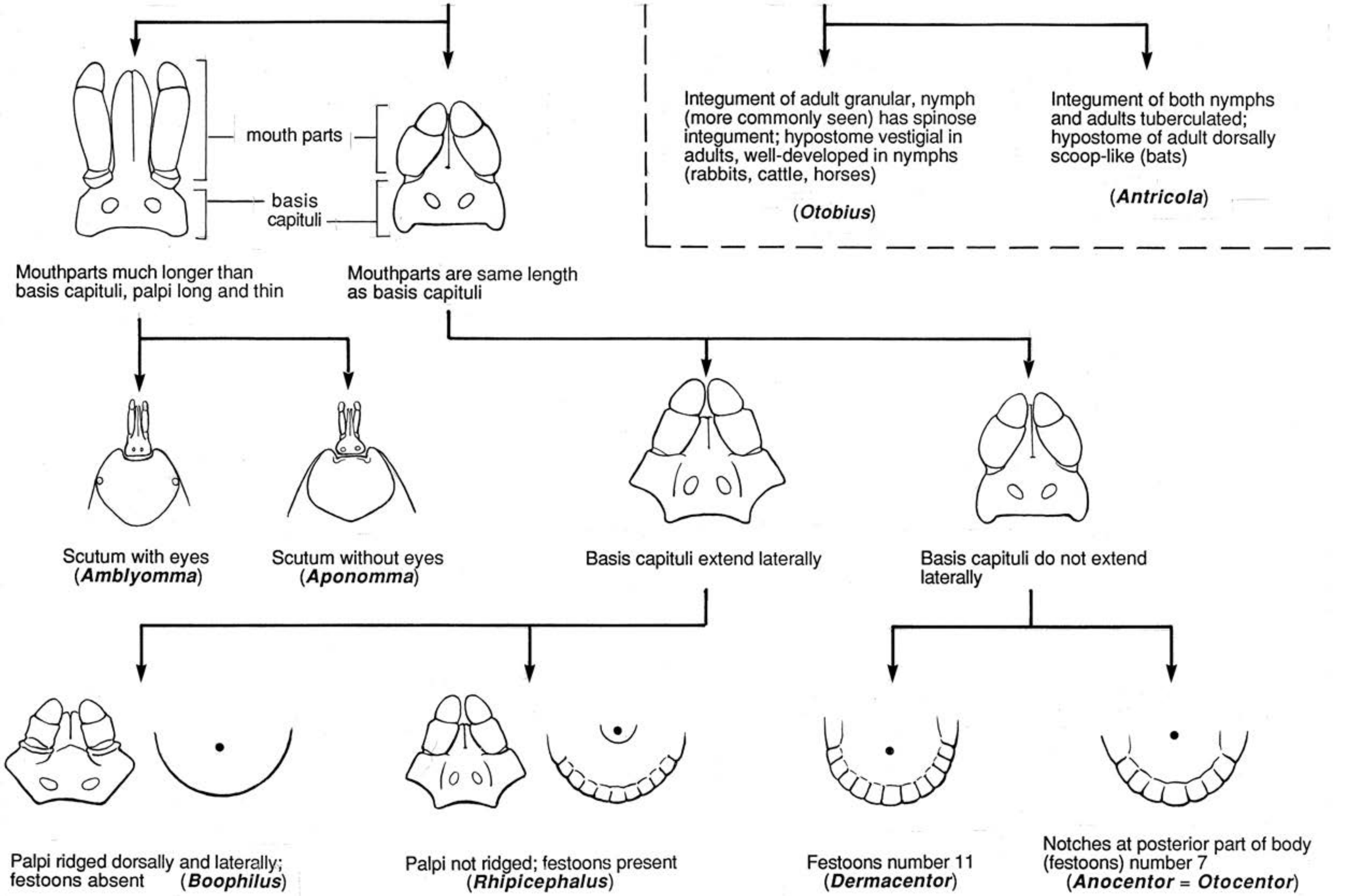

mouth parts
basis capituli
Mouthparts much longer than basis capituli, palpi long and thin
Mouthparts are same length as basis capituli
Integument of adult granular, nymph (more commonly seen) has spinose integument; hypostome vestigial in adults, well-developed in nymphs (rabbits, cattle, horses)
(*Otobius*)
Integument of both nymphs and adults tuberculated; hypostome of adult dorsally scoop-like (bats)
(*Antricola*)
Scutum with eyes (*Amblyomma*)
Scutum without eyes (*Aponomma*)
Basis capituli extend laterally
Basis capituli do not extend laterally
Palpi ridged dorsally and laterally; festoons absent (*Boophilus*)
Palpi not ridged; festoons present (*Rhipicephalus*)
Festoons number 11 (*Dermacentor*)
Notches at posterior part of body (festoons) number 7 (*Anocentor* = *Otocentor*)

TABLE 35.7 Differences between hard ticks and soft ticks

Feature	Hard Tick	Soft Tick
Scutum (dorsal shield)	Present	Absent, soft, leathery exoskeleton
Capitulum (mouth parts)	Anterior, visible from above	Ventral, not visible from above
Nymphal stages	One	Several
Adult feeding time	Several days	30–60 min
Female blood meals	One	Several
Egg-laying events	One	Several
Total eggs laid	3,000–8,000	400–500
Feeding habits	Feed several times during each stage	Feed only once during each stage
Feeding accommodation	Cuticle grows to accommodate large volume of blood ingested	Cuticle expands, but does not grow to accommodate large volume of blood ingested
Life span	Less than a year to >3 years	Several years
Genera	*Amblyomma*, *Dermacentor*, *Ixodes*, *Rhipicephalus*	*Argas*, *Antricola*, *Ornithodoros*, *Otobius*

TABLE 35.8 Descriptions of some of the more common hard ticks found in the United States

Tick	Description	Comments
Amblyomma americanum (Lone Star tick)	Reddish-brown tick species; adult stages have long mouth parts visible from above; adult females have distinct white spot on their back (scutum); males have no single spot but have inverted horseshoe-shaped markings at the posterior edge of their dorsal side	Central Texas east to the Atlantic; north to Iowa and New York; northern Mexico; probably most aggressive and common tick in southern United States; transmits tularemia, possibly ehrlichiosis
Amblyomma maculatum (Gulf Coast tick)	Somewhat similar to American dog tick, except with metallic instead of white markings, long mouth parts; adult females with metallic white or gold markings on scutum, males with numerous, mostly connected linear spots of golden white	Portions of Atlantic and Gulf Coast areas (100–200 miles inland, south into Mexico); increasingly a pest in southern United States; nuisance only
Dermacentor andersoni (Rocky Mountain wood tick)	Adults have shorter mouth parts than *Amblyomma*; usually dark brown or black with bright white markings on the scutum	Western counties of Nebraska and Black Hills of South Dakota to Cascade and Sierra Nevada Mountains; northern Arizona and northern New Mexico to British Columbia, Alberta, and Saskatchewan; transmits RMSF,[a] Colorado tick fever, tularemia; tick paralysis in United States and Canada
Dermacentor variabilis (American dog tick)	Adults dark brown or black; short, rounded mouth parts; dull or bright white markings on scutum	Medically important; primary vector of RMSF in the central and eastern United States; transmits tularemia, maybe tick paralysis; deticking dogs may lead to infection
Ixodes pacificus (western black-legged tick)	No white markings on dorsal side, no eyes or festoons, adults dark brown; moderately long mouth parts; appears almost identical to *I. scapularis*	Along Pacific coastal margins of British Columbia and the United States, possibly extending into Baja California and Mexico; most cases of Lyme disease in California are transmitted by this tick
Ixodes scapularis (black-legged tick)	Adults have no eyes, festoons, or white markings on dorsal side; dark brown but may appear light brown or orangeish	Northern form in New England states and New York, south into New Jersey, Virginia, and Maryland; upper Midwest and Ontario; southern form around southern Atlantic Coast states, Texas, Oklahoma, and Mexico
Rhipicephalus sanguineus (brown dog tick)	Light to dark brown, no white markings on dorsal side; have both festoons and eyes	Most widely distributed tick; almost worldwide in Western Hemisphere; transmits RMSF; found indoors around pet bedding areas; strong tendency to crawl upward (walls of infested houses)

[a] RMSF, Rocky Mountain spotted fever.

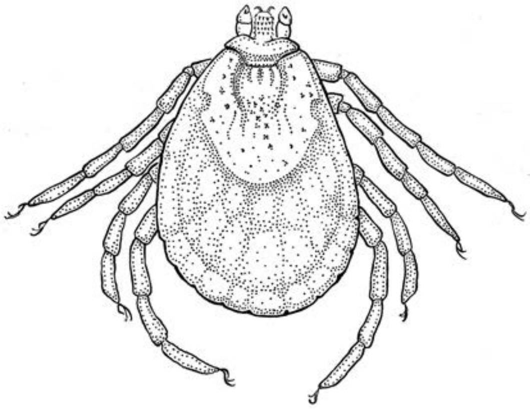


Figure 35.40 Hard tick, 4 to 10 mm. (Illustration by Sharon Belkin, based on an illustration from Najarian HH, *Textbook of Medical Parasitology*, The Williams & Wilkins Co, Baltimore, MD, 1967.) doi:10.1128/9781555819002.ch35.f40

about 1.5%. Studies looking at the prevalence of anti-arthropod saliva antibodies among residents in a high-risk community revealed a 79% positive rate compared with control groups (62). In the San Francisco Bay Area, the figure was 36%. In the study group, seropositivities for *I. pacificus* and for *B. burgdorferi* were significantly correlated. Experimental and field evaluations of two acaricides for control of *I. pacificus* in northern California appear very promising, and these substances can be a beneficial component of an overall control program (63).

The morphologic differences between *I. pacificus* and *I. scapularis* are minimal (Fig. 35.42 and 35.43). Both species have no white markings on their dorsal side and no eyes or festoons. Adults are dark brown, and the mouthparts are moderately long. Occasionally, *I. scapularis* appears light brown or orangish from the dorsal view (64). The difference between the nymph and the adult can be seen in Fig. 35.44. Descriptions of the more common ticks are given in Table 35.6.

Tick Removal. The proper way to remove a soft tick is to use a pair of curved tweezers. Wash your hands and put on a pair of disposable gloves. Grasp the tick at the base closest to the skin with the tweezers tips. Slowly pull the tick out, making sure not to yank, squeeze or twist it. Twisting or turning the tick does not make removal easier because the mouthparts are barbed. If the head of the tick remains embedded in the skin, apply a warm compress to help draw it out. Seek medical assistance if you cannot remove all the tick's parts. Wash the site with soap and water or a mild disinfectant. Do not cover the soft tick with rubbing alcohol, clear nail polish, or lighter fluid before removing it, and do not burn the tick with a match. These methods are dangerous and ineffective; worse, they can actually cause the tick to regurgitate into the body, leading to a greater chance of infection and disease transmission. Pending any medical problems or symptoms of a tick-borne illness, it is recommended that the tick be saved in a plastic bag or vial, stored in the freezer, and saved for several months.

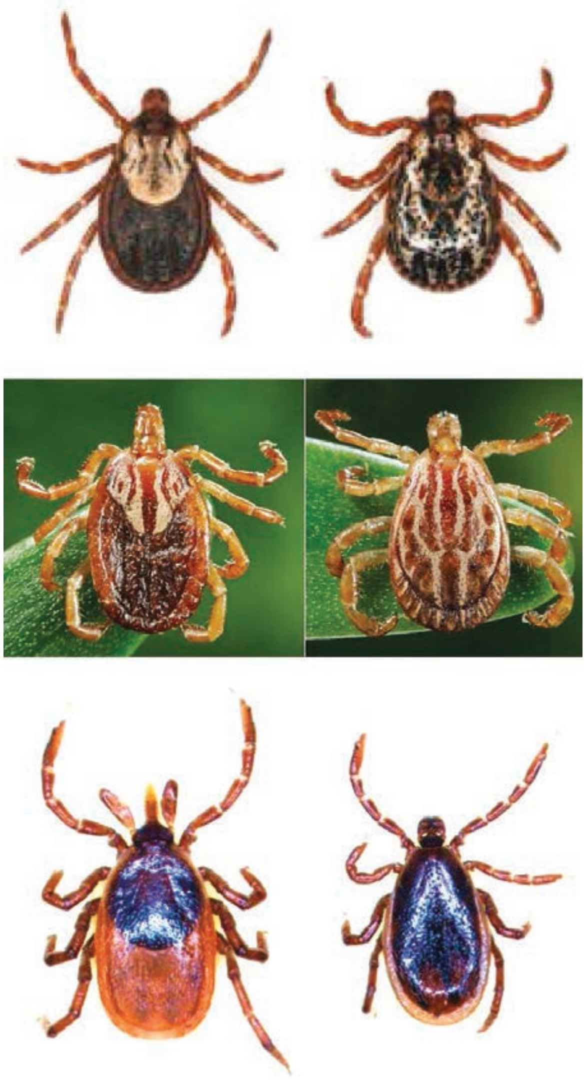


Figure 35.41 Hard ticks: (left) female; (right) male. (Top) *Dermacentor variabilis*, American dog tick. (Middle) *Amblyomma triste*, Argentina. (Bottom) *Ixodes scapularis*. (Courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch35.f41

Itch Mites (Mange Mites), Follicle Mites, and Dust Mites

Itch Mite. Dating back to over 2,500 years ago, scabies was known from the time of the Greeks and Romans through the Middle Ages as the "itch." Although the disease was acknowledged by numerous cultures over several eras, the cause of the infliction was never clearly deter-

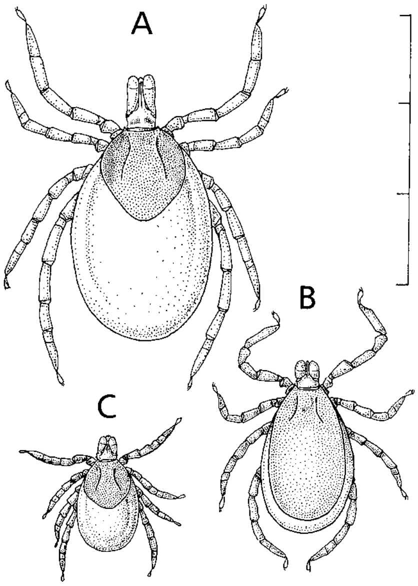


Figure 35.42 *Ixodes* spp. Diagram of the female (A) and male (B) adult ticks and the nymph (C) (bar, 3 mm). (Illustration by Sharon Belkin, based on information in reference 58.) doi:10.1128/9781555819002.ch35.f42

mined until Giovanni Cosimo Bonomo accomplished this in 1687. Several genera infect the skin of mammals, with *Sarcoptes scabiei* being found in humans, although some mange mites of animals occasionally cause a pruritic rash in humans (65). *S. scabiei* is microscopic and lives in cutaneous burrows, where the fertilized female deposits eggs (Fig. 35.45 through 35.49). Scabies is transmitted by close contact with infested individuals, including touching, shaking hands, sexual contact, and contact in day care centers with children and the elderly. Scabies is common among those in overcrowded conditions and has been associated with immigrants, poor hygiene, poor nutritional status, homelessness, dementia, and sexual contact.The usual skin sites that are susceptible to infection are the interdigital spaces, backs of the hands, elbows, axillae, groin, breasts, umbilicus, penis, shoulder blades, small of the back, and buttocks (Fig. 35.46 through 35.48) (9). The outstanding clinical symptom is intense itching. Scratching commonly causes weeping and bleeding and sometimes leads to secondary infection. A form of the infestation, called crusted scabies, can occur in immunosuppressed or anergic individuals; many mites are present in keratotic excrescences on the body and extremities, but pruritus is usually absent (Fig. 35.48). This infestation is highly contagious and has been reported to be the cause of hospital epidemics. Scabies occurs worldwide, with at least 300 million cases each year. Oral ivermectin, at a dose of 300 µg/kg, given as a single dose and repeated after 7 days, proved effective for the treatment and prophylaxis of scabies in an infected institutional environment (66).

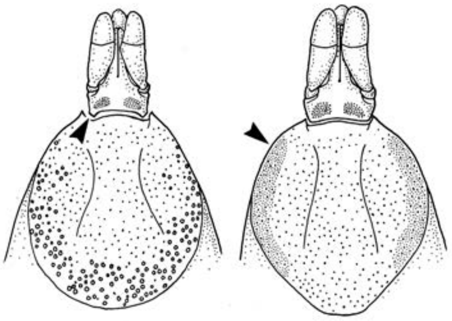


Figure 35.43 (Left) *Ixodes scapularis*. The scutum (the dorsal shield, which is small in the female and almost covers the dorsal surface in the male) and the terminal capitulum, which is attached to the anterior end of the body of *Ixodidae*. Note that the scutum is nearly circular, with punctuations larger peripherally. The cornua (arrowhead) is small but definite for *I. scapularis*. (Right) *Ixodes pacificus*. Scutum and terminal capitulum. Note that the scutum is oval with uniformly distributed small punctuations (arrowhead), and the cornua is absent. (Illustration by Sharon Belkin, based on information in reference 58.) doi:10.1128/9781555819002.ch35.f43

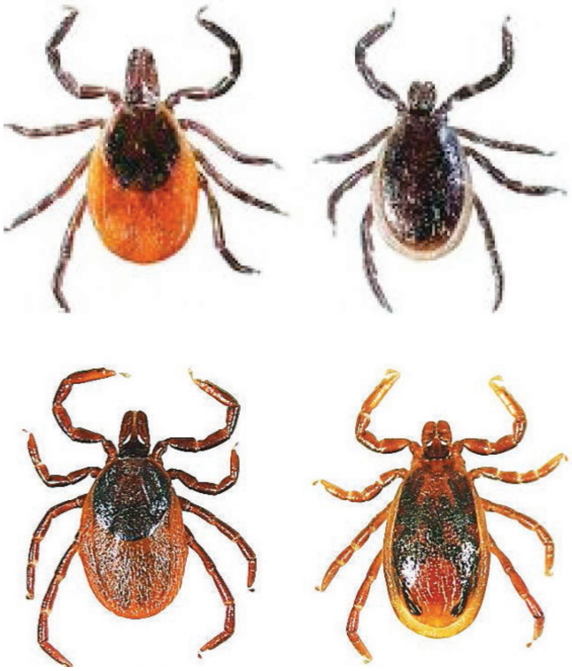


Figure 35.44 (Upper) *Ixodes scapularis*: (left) adult female; (right) adult male. Note that there are no white markings on the dorsal surface and no eyes or festoons (Table 35.6). (Lower) *Ixodes pacificus*: (left) adult female; (right) adult male. Note the mouthparts are almost identical, but color variation is less common with *I. scapularis*. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch35.f44

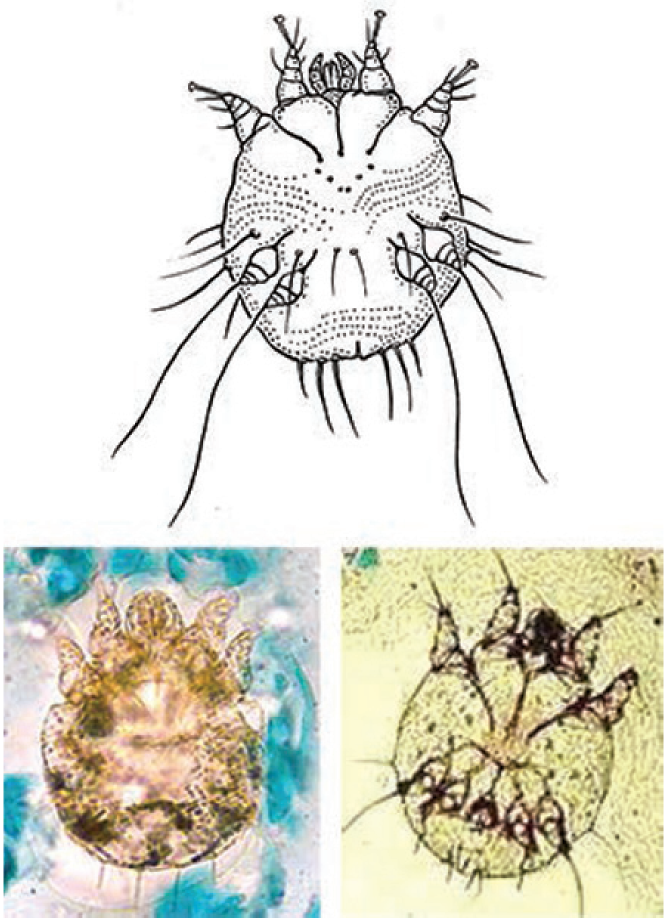


Figure 35.45 Itch mite (*Sarcoptes* sp.). (Top) Adult mite, ventral surface, ~0.25 mm (illustration by Sharon Belkin, based on an illustration from Najarian HH, *Textbook of Medical Parasitology*, The Williams & Wilkins Co, Baltimore, MD, 1967). (Bottom, left) Adult mite, dorsal surface; (right) adult mite, ventral surface; note the legs are now visible. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch35.f45

The most common symptoms are produced by the host immune reaction to burrowed mites and their products. Pruritus and rash may take up to 2 to 6+ weeks to develop after initial exposure to the scabies mite. For this reason, the early clinical presentation of scabies should be detected as soon as possible to prevent the spread of infestation. Skin scraping with dermoscopy is implicated as the diagnostic method of choice for scabies at the present time. Dermoscopy is especially useful in diagnosis of incognito scabies (67). In addition, the presence of visible burrows could be a reliable positive marker of scabies in the absence of dermoscopy or microscopy data. Specific techniques for recovery of the mites are listed below.

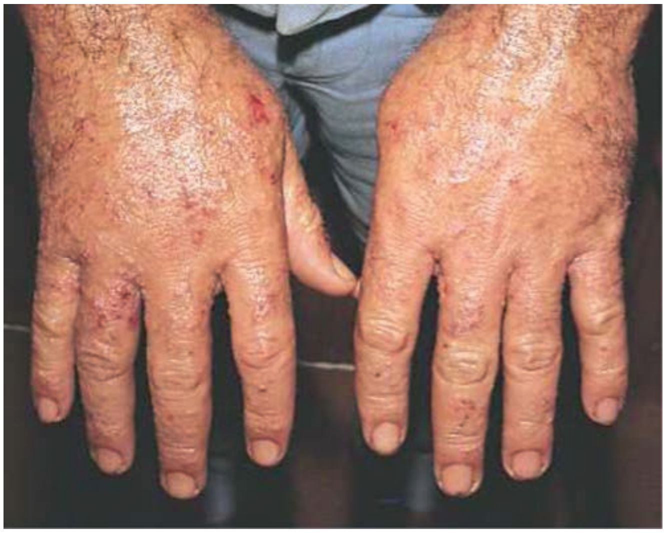
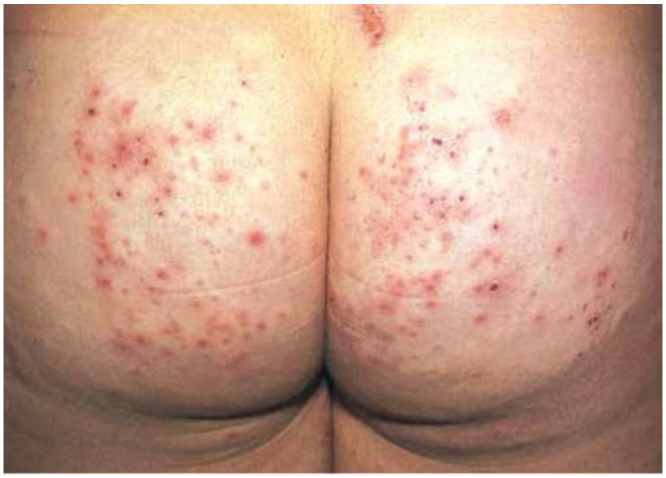


Figure 35.46 Scabies of hand. Lesions are complicated by scratching and secondary bacterial infection. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch35.f46

Skin-scraping technique. The diagnosis can be confirmed by demonstration of the mites, eggs, or scybala (fecal pellets). Because the mites are located under the surface of the skin, scrapings must be made from the infected area.

1. Place a drop of mineral oil on a sterile scalpel blade. (Mineral oil is preferred to potassium hydroxide solution or water. Mites adhere to the oil, skin scales mix with the oil, the refractility differences are greater between the mite and the oil, and the oil does not dissolve fecal pellets.)
2. Allow some of the oil to flow onto the papule.

Figure 35.47 Scabies of buttocks of a Brazilian child. There is evidence of scratching and secondary bacterial infection. (Armed Forces Institute of Pathology photograph.)
doi:10.1128/9781555819002.ch35.f47



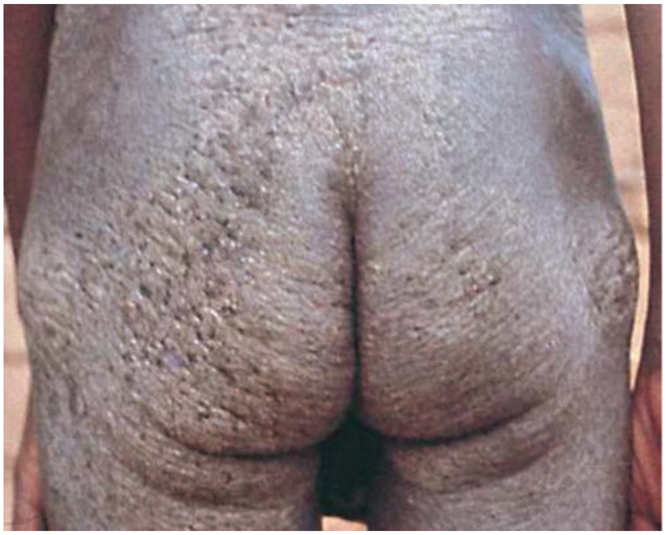
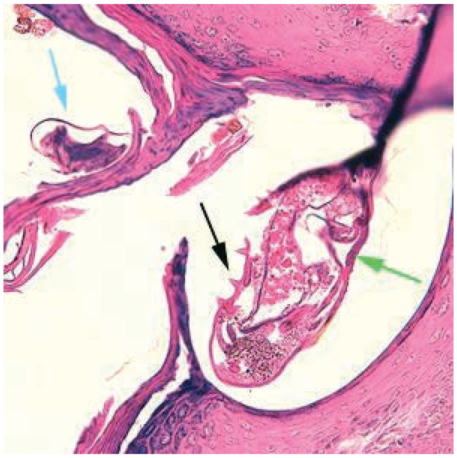


Figure 35.48 Crusted scabies over the buttocks of a 19-year-old Congolese man. The patient was chronically ill, suggesting immunosuppression. Lesions are papulosquamous and excoriated. Scaling and crusting were severe about the elbows and from the lower trunk to the knees. (Armed Forces Institute of Pathology photograph.) doi:10.1128/9781555819002.ch35.f48

3. Scrape vigorously six or seven times to remove the top of the papule. (There should be tiny flecks of blood in the oil.)
4. Transfer the oil and scraped material to a glass slide (an applicator stick can be used).
5. Add 1 or 2 extra drops of mineral oil to the slide and stir the mixture. Any large clumps can be crushed to expose hidden mites.
6. Place a coverslip on the slide and examine (first on low power). The adult mites range from approximately 215 to 390 μm in length, depending on sex. The eggs are 170 μm long by 92 μm wide, and the fecal pellets are about 30 by 15 μm. The fecal pellets are yellow-brown.

Figure 35.49 *Sarcoptes scabiei* mites in a skin biopsy, stained with hematoxylin and eosin. An adult (green arrow) and an egg (blue arrow) are visible. Note the presence of cuticular spines (black arrow) on the adult. (Courtesy of the CDC Public Health Image Library.) doi:10.1128/9781555819002.ch35.f49



Plastic box or petri dish method. If mineral oil preparations of skin scrapings fail to demonstrate the mites, the encrusted skin scrapings, etc., can be placed in a small plastic box or small petri dish. The container should be left undisturbed at room temperature for 12 to 24 h. Away from the living host, the mites drop to the bottom of the box or dish and can be seen with a magnifying glass or dissecting microscope.

Follicle Mites. Two species of follicle mites parasitize humans; each has its own particular habitat. *Demodex folliculorum* (294 μm by 55 μm) is found in the hair follicles above the sebaceous glands, and *Demodex brevis* (208 μm by 54 μm) occurs in the sebaceous glands. Both are found in the epidermis of the nose, nasolabial fold, forehead, and adjacent regions of the face; these mites have also been associated with dermatitis of the scalp. These follicle mites can be considered more of a nuisance than a true pathogen and are considered to be harmless saprophytes; symptoms may include a mild rosaceous pruritus and a fibrous tissue response around the mites. *Demodex* sp. has been associated with folliculitis, pityriasis, rosacea, blepharitis, and seborrheic dermatitis. Immunosuppressed patients may develop extensive cutaneous lesions (68).

Estimates of human infection range from 25 to 100%. The two species have similar morphology, with *D. brevis* being somewhat shorter. They are elongated wormlike mites with rudimentary legs (Fig. 35.50 and 35.51). Most humans acquire mites early in life, often by mother-to-child transmission. Adults of both species are tiny, elongate, and vermiform; they have three body divisions: the gnathosoma (short anterior region that contains the mouthparts), podosoma (has four pairs of short stumpy legs), and opisthosoma (long posterior half of the body that tapers to a rounded end).

House Dust Mites. Although house dust mites in the genus *Dermatophagoides* are not commonly seen or submitted to the laboratory for confirmation, it is important to realize that these mites are directly linked to allergic reactivity that can be detected in patients with atopic eczema. Powerful allergens can be found in the mites *D. pteronyssinus* and *D. farinae*, as well as in their fecal pellets and secretions. Allergic rhinitis, asthma, and childhood eczema are attributable to these mites. The mites feed on shed human skin scales, but they also eat mold, fungal spores, pollen grains,

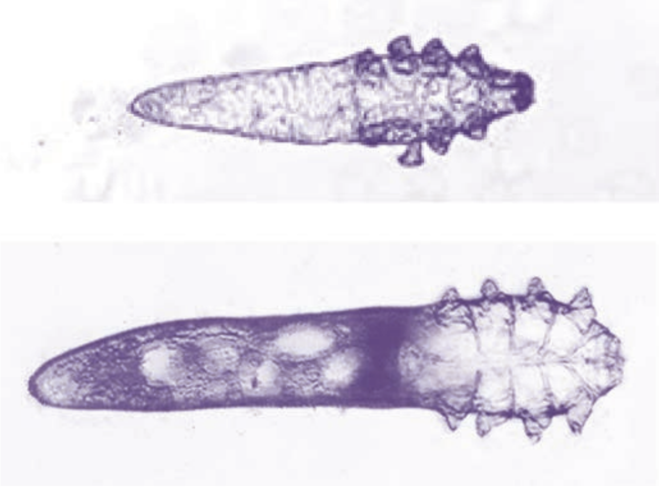


Figure 35.50 Follicle mites. (Upper) *Demodex brevis*; (lower) *D. folliculorum*. Note the rudimentary legs.
doi:10.1128/9781555819002.ch35.f50

feathers, and animal dander. Active treatment includes Gore-Tex bags for all bedding elements such as the mattress and box springs, a high-power vacuum cleaner, and a spray containing benzyl alcohol and tannic acid to kill mites and denature allergens. These measures are highly effective, particularly the Gore-Tex bed bags (69). This approach was associated with clinical improvement in most patients with atopic eczema, particularly those who were the most severely affected. However, some of the acaricide applications are required at 2- to 3-month intervals to maintain optimal effectiveness (70). Also, treatments are different for different allergens (71). Soft toys are a major source of house dust mites and their allergens, and sleeping with soft toys is a significant risk factor for subsequent sensitization. Treating soft toys by freezing, hot tumble drying, or washing with eucalyptus oil (0.2% to 0.4%) resulted in significant reductions in live mites (72).

Figure 35.51 Section of sebaceous gland showing *Demodex* sp. in hair follicle. Note the short anterior gnathosoma (gn), podosoma with four pairs of legs (po), and long posterior opisthosoma (op). (Armed Forces Institute of Pathology photograph.)
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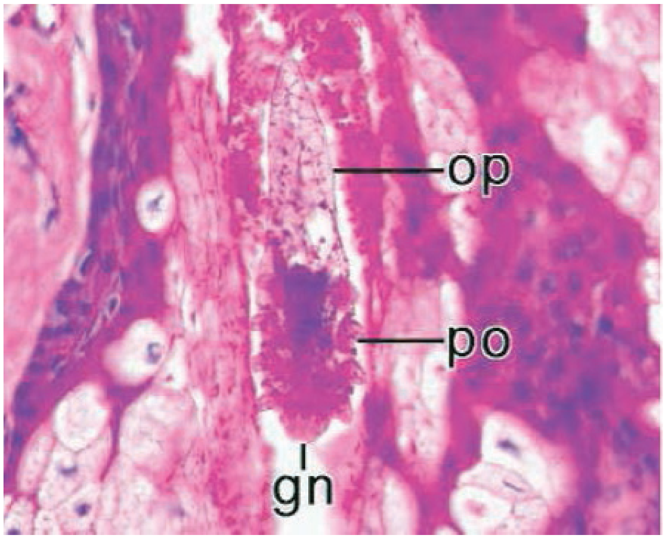

Subclass Araneae (Spiders)

The subclass Araneae contains the spiders, only a few of which can cause pathologic changes in humans. All true spiders produce venom, although few types of venom cause more than a local irritation at the bite site. Those that can cause tissue damage are categorized as causing systemic arachnidism or primarily necrotic arachnidism.

Systemic Arachnidism (Black Widow, Other Widow Spiders)

Widow Spiders. The black widow spider (*Latrodectus mactans* and other species) has the typical shiny black abdomen with a red hourglass shape on the ventral surface (Fig. 35.52). However, it is important to realize that the typical hourglass-shaped marking on the underside of the abdomen may not look like the textbook description, and there are at least five species of "widow" spiders within the United States. Some black widow spiders have bright red hash marks on the dorsal surface in addition to the red hourglass on the underside. Also, the hourglass marking may appear "broken" and not complete (6). There may also be red or white marks on the dorsal side of the abdomen. The color patterns on the underside of the abdomen can vary quite a bit, with what appear to be dots, stripes, or a combination of the two. The immature spiders are tan to gray with little to no black color.

Black widow spiders can cause variable clinical sequelae from local damage to very serious conditions including death. The males and immature black widow spiders bite, but symptoms are mild due to the smaller amount of venom injected. The major component of the venom is alpha-latrotoxin, a protein that causes catecholamine release at adrenergic nerve endings and acetylcholine depletion at motor nerve endings (73). The nervous system is the primary target of alpha-latrotoxin; however, other tissues, such as the heart and lungs, are also susceptible to its toxic effect. Although serious systemic sequelae (abdominal cramps, hypertension, reduced heartbeat and feeble pulse, convulsions, shock, delirium, and death) can occur, uncomplicated recovery is the usual outcome (weakness, myalgia). Following recovery, the individual usually develops complete systemic immunity to subsequent bites, although there may be a local reaction. At the bite site, two small

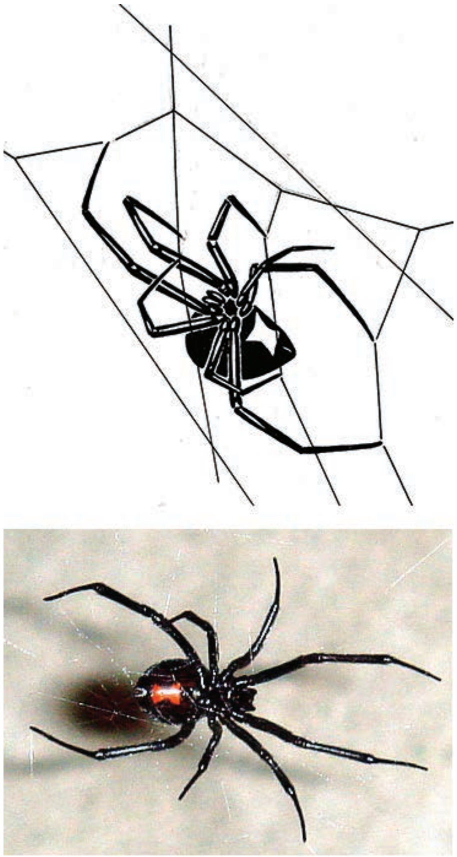

Figure 35.52 Black widow spider (*Latrodectus* sp.), adult, 20 mm. (Upper, illustration by Sharon Belkin; lower, courtesy of the CDC Public Health Image Library.)
doi:10.1128/9781555819002.ch35.f52

red spots may be visible, representing the skin penetration sites.

The wound should be cleaned and ice packs should be applied to limit the spread of the venom. In cases of more severe reactions, the patient should seek medical attention; the use of intravenous solutions, antivenom, and tetanus vaccine are relevant considerations. Children, the elderly, and patients with underlying medical conditions may require hospitalization. In a retrospective study of adverse events following black widow antivenom, results suggest that Black Widow Spider Antivenin (Merck) administration is relatively safe, with mild to moderate adverse effects seen in only a small percentage of patients. There were no deaths or severe allergic reactions identified.

Black widow spiders tend to avoid strong light and prefer dark areas behind or underneath appliances, furniture, closets, etc. These spiders are very reclusive and will not bite unless provoked; generally, they attempt to escape. They are more active in the summer months. After mating, the female kills the male only if she is not well fed, thus ensuring the survival of the eggs; as many as 2,000 offspring may be produced in a single year.

Necrotic Arachnidism (Violin or Brown Recluse Spider, Hobo Spider, Funnel Web Spider, Tarantula Spider)

Violin Spiders. *Loxosceles reclusa*, commonly referred to as the brown recluse or violin spider, is a venomous spider endemic to the southeastern and midwestern United States. *L. reclusa* (and other species) is commonly found inside human habitation rather than in the outdoor areas where the black widow spider can be found (as well as indoors) (74). These spiders are of medium size (0.9 to 1.5 cm long), are yellowish to dark brown, and tend to have a fiddle-shaped marking on the dorsal surface (Fig. 35.53).

Brown recluse spider bites are most often benign and self-limiting, but in a few cases can cause severe necrotic skin lesions (75). Geographical location should be considered when a brown recluse spider bite is suspected; there have been reports of physicians misdiagnosing these bites in areas where *L. reclusa* is not known to be endemic (76). One of the signs of a brown recluse spider bite is the tendency of the lesion to extend downward; the direction of the lesion depends on the patient's position as vessel

Figure 35.53 Violin spider (*Loxosceles* sp.). (Upper) Violin/brown recluse spider, 9 to 15 mm (illustration by Sharon Belkin). (Lower) Note the violin image in the head area (arrow). (Armed Forces Institute of Pathology photograph.)
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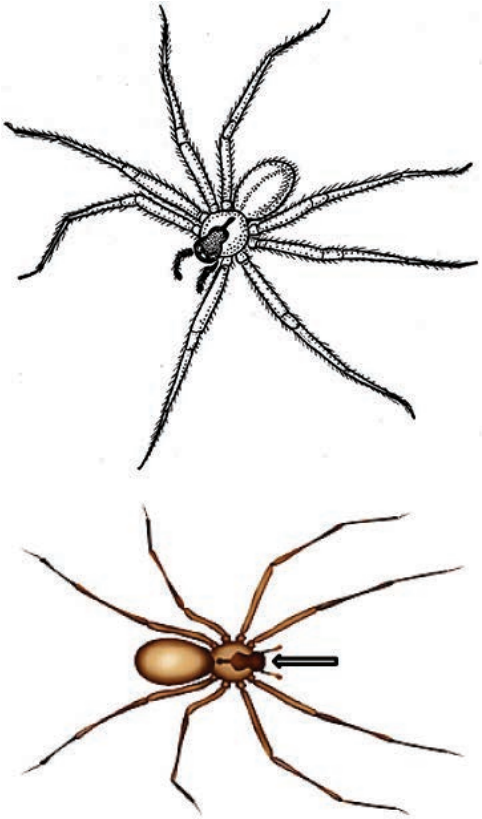


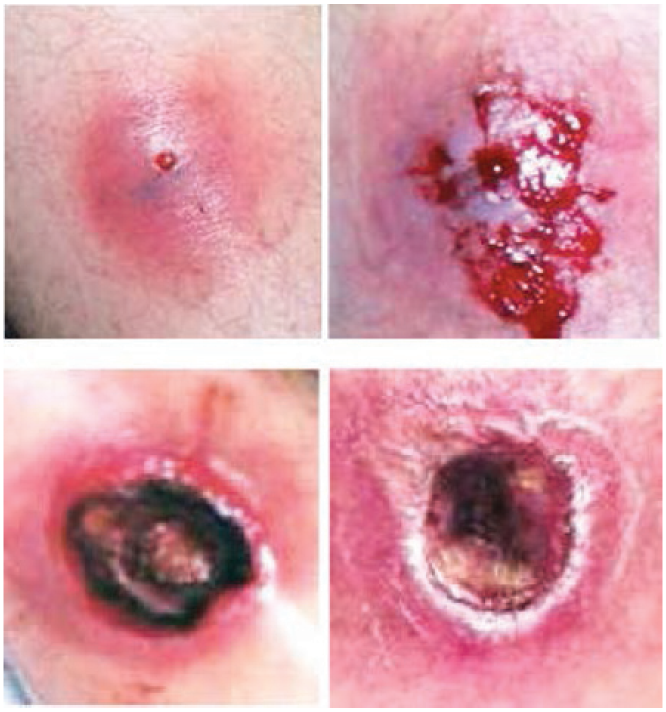


Figure 35.54 Tissue necrosis caused by *Loxosceles* sp. (brown recluse, violin spider); note the progression of the wound from day 1 to approximately the end of the year (upper left to lower right). doi:10.1128/9781555819002.ch35.f54

damage occurs. This bite presentation is apparently rare with other arthropod bites. Mild to severe pain can occur immediately after the bite or within a few hours. Extensive tissue necrosis can occur, with extended spread, very slow healing, and severe disfigurement (Fig. 35.54). If a large dose of venom is injected, there may also be systemic complications, such as hemolytic anemia, hemoglobinuria, hematuria, jaundice, and fever. There is a high mortality rate associated with systemic complications, which can include hematuria, anemia, fever, rash, nausea, vomiting, coma, and cyanosis. If systemic symptoms occur, they usually appear several days after the bite; as an example, hemolysis has been documented to occur from 24 h to 3 days after a bite.

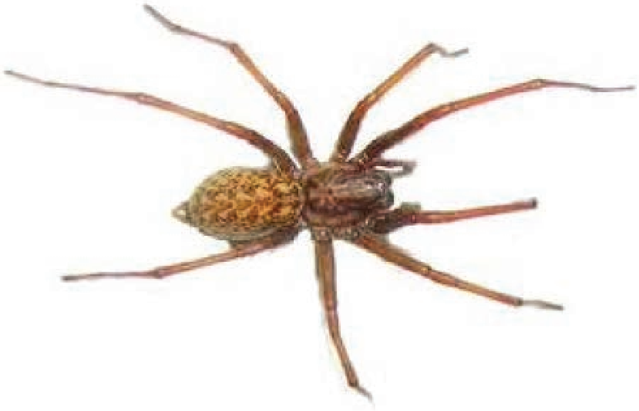
The brown recluse is nocturnal and is generally found in bathrooms, bedrooms, cellars, attics, clothing folds, boxes, storage areas, under rubble, etc. Like the black widow spider, it is not aggressive but will bite when bothered. As of 2011, the range of *L. reclusa* is centered in the United States, from Kansas east to Kentucky and from southern Iowa south to Louisiana. Newly influenced areas may include parts of Nebraska, Minnesota, Wisconsin, Michigan, South Dakota, Ohio, and Pennsylvania (77).

In South America, there is a close relative of the brown recluse, *Loxosceles laeta*, which also causes a severe necrotic lesion; these spiders have been introduced and are now well established in Los Angeles, CA, and at Harvard University in Cambridge, MA (6). Other species found mostly in the Southwest include *L. refescens*, *L. deserta*, *L. devia*, and *L. arizonica*.

As with any spider bite, if possible the spider should be collected and submitted for identification. In the case of the brown recluse, prompt medical attention is recommended. Although a specific antivenom has been used, it is not generally available. Treatment is somewhat controversial, ranging from ice packs to complete surgical excision of the bite site. Antibiotics such as cephalosporin or erythromycin have been used, as well as dapsone; however, the side effects of dapsone may also mimic symptoms resulting from the spider bite. Even in cases of verified spider bite, differentiation must be made between toxic effects of venom and secondary infections, such as incurred when pruritus leads to scratching. The medical community needs to be aware of the many causes of necrotic wounds that can be misdiagnosed as recluse bites. Culture of specimens taken from wounds might accurately determine the plethora of causative agents of so-called brown recluse bites, which can vary from being arthropod, bacterial, viral, or fungal in nature or due to underlying disease states.

Hobo Spiders. Hobo spiders are large, fast spiders, about 45 mm long with distinct chevron stripes on the abdomen. They are light to medium brown color, the thorax has two dark stripes and one inner light stripe, and the abdomen has a light stripe down the midline that is interrupted by light markings (Fig. 35.55). They build funnel-shaped webs in or around houses. They are also now recognized as causing necrotic lesions, particularly the males, which are responsible for most bites. Reports of bites in Washington, Oregon, and Idaho continue to increase. Although the bites were originally thought to be from brown recluse spiders,

Figure 35.55 Hobo spider, 45 mm long with distinct chevron stripes on the abdomen. doi:10.1128/9781555819002.ch35.f55



this was never confirmed. It appears that the bite of these hobo spiders can cause lesions similar to those seen with bites by the brown recluse. The species involved is *Tegenaria agrestis*. Although the bite is usually painless at first, the same types of lesions and symptoms occur as seen with bites by the brown recluse. Some of these lesions can take years to heal, and systemic symptoms can also be seen. The bite of the hobo spider usually is initially painless. Induration may appear within 30 minutes, surrounded by an area of expanding erythema that can attain a diameter of 5 to 15 cm. Blisters develop within 15 to 35 hours; the blisters can rupture with a serous exudate encrusting the cratered wound. An eschar can develop with underlying necrosis and eventual sloughing of affected tissue. Lesions normally heal within 45 days, but can result in a permanent scar; healing may take 3 years if the bite occurred in fatty tissue. The most common symptom is a severe headache, which occurs as soon as 30 minutes after the bite and usually within 10 hours, and can persist for a week. Other symptoms can include nausea, weakness, fatigue, temporary memory loss, and vision impairment. Protracted systemic effects, including aplastic anemia, intractable vomiting, or profuse secretory diarrhea, are rare but may be associated with death.

Funnel Web Spiders. Funnel web spiders are large, dark-colored, and very aggressive; they have prominent fangs and are found in and around Sydney, Australia. They are glossy dark brown to black, while the abdomen is dark plum to black; including the legs, they measure 45 to 60 mm (Fig. 35.56). They tend to build funnel webs in burrows, rotting logs, tree holes, or rock crevices; colonies can number more than 100 spiders. These are very dangerous spiders, and the bite can cause death in as little as 15 min. The bite can become very painful, and systemic symptoms such as hypotension and apnea can begin to occur within just a few minutes.

It is important for first aid to be administered immediately, including a pressure bandage and immobilization of the limb with splints. Patients are usually hospitalized and monitored for signs and symptoms indicating that venom has or has not been injected with the bite. These systemic symptoms can include mouth numbness, tongue spasms, nausea, vomiting, profuse sweating, salivation, and muscle spasms. Appropriate administration of antivenom is indicated; however, it is important to carefully monitor patients for possible allergic reactions to the antivenom.

Tarantula Spiders. Tarantulas occur throughout different areas of the world and become aggressive only if provoked or handled inappropriately. They are large, hairy spiders (Fig. 35.57). The 30 species found in the southwestern United States have a leg span of approximately 18 cm, while the South American species found in the jungles measure 24 cm. Bites can vary from being relatively painless to causing a deep, throbbing pain that may last for hours. Individuals who are hypersensitive may present with more serious symptoms. Tarantulas sold for pets within the United States are generally species of *Abhonopelma*, which tend to be very colorful. Bites by several genera of African, Asian, and Australasian tarantulas can cause

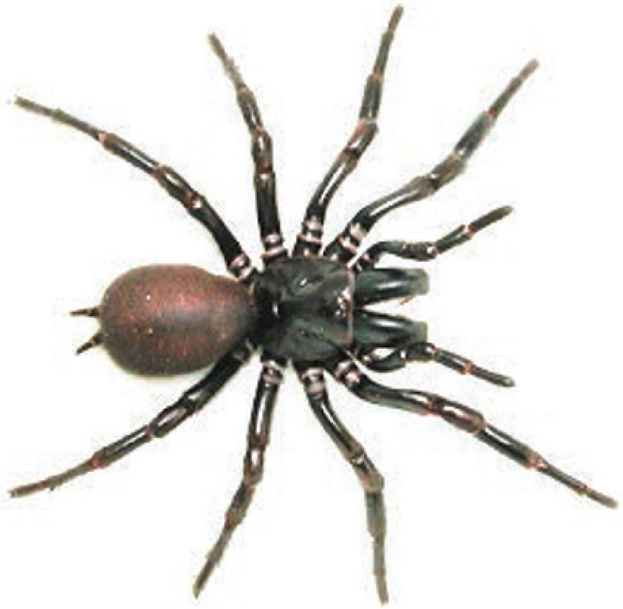
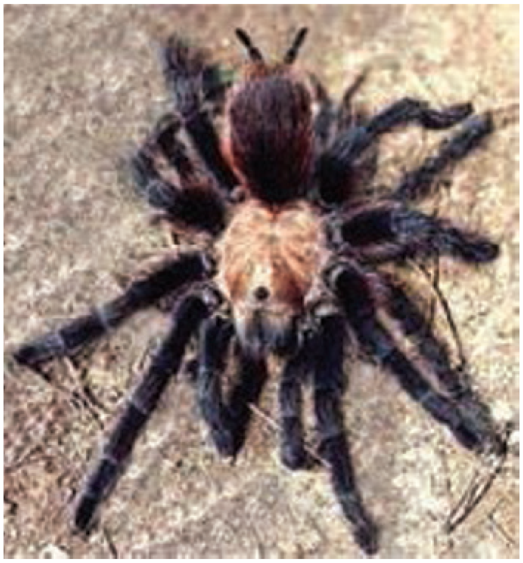


Figure 35.56 Funnel web spider, 45 to 60 mm, large, dark colored. (Courtesy of Museum Victoria, photographer Alan Henderson, www.museumvictoria.com.au.)
doi:10.1128/9781555819002.ch35.f56

Figure 35.57 Tarantula (note the hairy legs). (Courtesy of the U.S. Department of Agriculture.)
doi:10.1128/9781555819002.ch35.f57

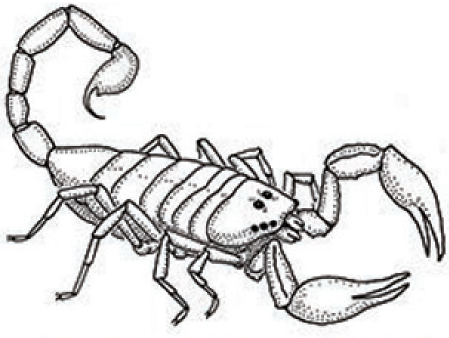


systemic neurotoxic envenoming. In the absence of available antivenom, severe persistent muscle spasms, reminiscent of latrodectism, pose a serious therapeutic challenge. New World tarantula bites appear to be clinically trivial with the possible exception of a few genera such as *Sericopelma* (*Theraphosinae*). However, Old World tarantulas, which lack protective urticating hairs, are more ready to bite and commonly cause local pain, swelling, and stiffness of the joints.

Subclass Scorpiones (Scorpions)

Scorpions are characterized by having large pedipalps that end in stout claws and a tail ending in a hooked stinger (Fig. 35.58). Venom is discharged through the stinger. Humans usually come into contact with scorpions by stepping on them or touching them in the dark. Species considered to be dangerous occur throughout the world. Some species can even penetrate the soles of the feet and inject venom, causing symptoms which can be serious, particularly in children younger than 5 years (aching pain radiating from the site; lymphadenitis; generalized numbness; throbbing and twitching of fingers, toes, and earlobes; profuse sweating; glossopharyngeal involvement; vomiting; abdominal muscle spasms; convulsions; mental disturbances; partial paralysis; blindness; electrocardiographic changes; and death from respiratory paralysis).

Figure 35.58 Scorpion. (Upper) 30 to 40 mm (illustration by Sharon Belkin). (Lower) Although painful, scorpion stings are rarely fatal. Exceptions are infants and children who sustain multiple stings. The venom is contained in two venom glands in the tail. (Courtesy of the U.S. Department of Agriculture.) doi:10.1128/9781555819002.ch35.f58



Children younger than 5 years are most commonly exposed and have the most severe sequelae. It has been reported that in Trinidad, the death rate in exposed children can reach 25% (9). Usually, the death rate in exposed adults is very low.

Scorpions are nocturnal and generally are hidden during the day. Around homes, they are often found under houses or in attics. When actively seeking water, they may be found in kitchens or bathrooms, occasionally in the sink or bathtub. In the southwestern United States, the common striped scorpion, *Centruroides vittatus*, is found; it has a sting similar to that from a wasp or bee. *C. vittatus* is found in Texas, while *Centruroides exilicauda* is found in southern Arizona and western New Mexico.

Most stings are not serious, and the burning sensation and wheal disappear without any intervention. However, after a sting by one of the more dangerous species, it is important to minimize the spread of the venom. Ice packs are recommended, and medical treatment may be required. Areas of the world where dangerously venomous scorpions are found include (Old World) Algeria, Egypt, Iraq, Israel, Jordan, Morocco, South Africa, Sudan, and Turkey and (New World) Argentina, Brazil, Guyana, Mexico, Trinidad, United States (Arizona and New Mexico), and Venezuela (6).

Scorpion envenomations are a major public health problem in Brazil, whose most dangerous cases are attributable to the genus *Tityus*; approximately 50,000 cases are reported annually. Symptoms frequently observed at the sting site were local and radiating pain, paresthesia, edema, erythema, sweating, piloerectio, and burning. The systemic manifestations were significantly higher in patients from the western part of Brazil. Furthermore, neurological symptoms such as general paresthesia, ataxia, dysarthria, myoclonus, dysmetria, and electric shock-like sensations throughout the body were reported only by patients from the west (78). The specific treatment for scorpion envenomations in Brazil is based on the administration of species-specific antivenom, obtained from plasma of horses immunized with the venoms of *T. serrulatus*, which has been used effectively for the treatment of human scorpionism attributable to *T. serrulatus* and other species such as *T. stigmurus* and *T. bahiensis*.

Other Arthropods

Class Chilopoda (Centipedes)

Centipedes are usually not considered dangerous, and their importance is related to their occasional painful bites. These arthropods like to live in dark, damp environments such as debris and rubbish. They have a long, slender,

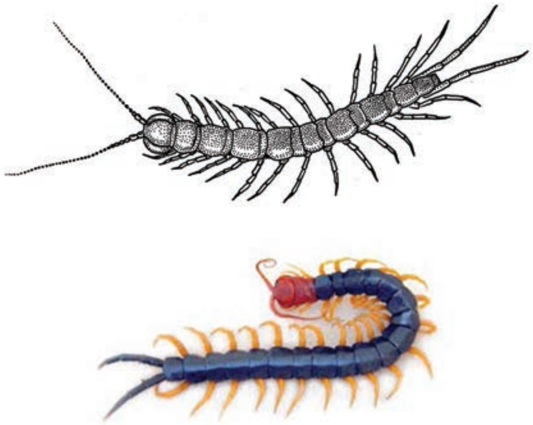


Figure 35.59 Centipede. (Upper) 30 to 40 mm; some may be much larger (illustration by Sharon Belkin).(Lower) *Scolopendra heros*. This is the largest-bodied centipede in North America, growing up to 7 inches or so. It ranges from Kansas and southern Missouri to Louisiana, westward through Texas and New Mexico/eastern Colorado to the Colorado River in Arizona, and southward about one-fourth of the way into Mexico. (Photograph courtesy of Seth Peterson, http://bugguide.net/node/view/50108.) doi:10.1128/9781555819002.ch35.f59

segmented body with a single pair of legs per segment except for the last two segments (Fig. 35.59) (6). The appendages of the first body segment are modified into poison claws. Although the poison injected is not harmful in the temperate areas of the world, the bite of *Scolopendra* spp. within the tropics and subtropics can be very painful and can cause edema, blistering, and necrotic lesions. In some cases, systemic reactions such as fever, nausea, vomiting, and headache also occur, particularly with *Scolopendra gigantea*. Rarely, some species have been recovered from the human nares and frontal sinuses or from the digestive tract; in each case, the centipede was an accidental invader of the human body (5).

Most bites are mild and self-limiting. The bite site should be thoroughly washed, with subsequent application of an ice pack and analgesics for pain. If the lesion becomes inflamed, antibiotics may be recommended.

Class Diplopoda (Millipedes)

Millipedes also have a long, slender, segmented body, but unlike the centipedes, they have two pairs of legs per segment in the abdominal region (Fig. 35.60). Also, they tend to be more round and “wormlike” in shape. They do not have piercing mouthparts, and they are not poisonous. However, some of the body secretions can result in a burning sensation, discoloration, and blister formation when applied to human skin. Severe conjunctivitis can also occur when the eyes come in contact with these fluids (55). Apparently, these secretions contain hydrogen cyanide as well as other aliphatic acids (9).

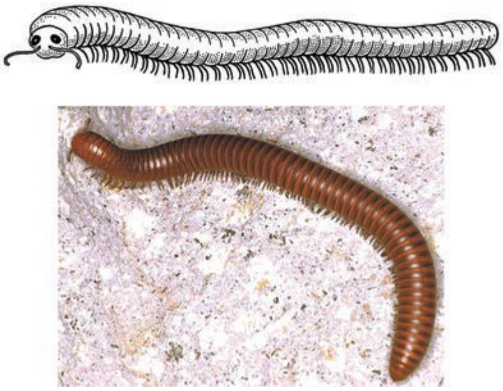


Figure 35.60 Millipede, 30 to 40 mm. (Upper, illustration by Sharon Belkin; lower, courtesy of the U.S. Department of Agriculture.) doi:10.1128/9781555819002.ch35.f60

Class Crustacea (Copepods, Crabs, Crayfish, etc.)

The crustaceans are primarily gill-breathing arthropods of both freshwater and saltwater. Only a few are of medical importance, and these tend to be found in freshwater. Some of the members of this group serve as intermediate hosts for various human helminth parasites. Copepods, or water fleas, serve as intermediate hosts for several helminth infections. Species of *Cyclops* carry the larval forms of *Dracunculus medinensis* (guinea worm), *Diphyllobothrium latum* (broad fish tapeworm), and *Gnathostoma spinigerum* (uncommon nematode infection). Species of *Diaptomus* are important first intermediate hosts of *D. latum*. Prevention of contamination and boiling or filtration of contaminated water will prevent these infections.

The larger crustaceans (decapods) include crabs and crayfish, which serve as the second intermediate hosts of *Paragonimus* spp. (lung fluke). Although the crabs incriminated in the transmission of *Paragonimus* spp. are freshwater forms, they can also survive in brackish water. Thorough cooking of crabs and crayfish will help eliminate some of these infections.

In the South Pacific, prawns may be paratenic hosts of the nematode *Angiostrongylus cantonensis*, which can cause eosinophilic meningitis and cerebral damage in the human host.

Control of Arthropods of Medical Importance

Measures for the control of medically important arthropods usually involve a number of different options, including physical control, biological control, and chemical control.

Although chemical means are generally the most widely used, they rarely succeed in providing a permanent solution to the problem. Often, a combination of methods provides the most effective control (79). A general dichotomous key for the identification of some of the arthropods can be found in Appendix 35.4, and a key to the important myiasis-producing larvae is provided in Appendix 35.5.

Physical Control

A number of control measures are related to the environment and may affect arthropod life cycles. An excellent example is demonstrated by the elimination of larval habitats of mosquitoes; this is basically water management. This approach could include rerouting of water, elimination of standing water, and ditch filling. With periodic monitoring, these should be long-term changes and should require little to no maintenance. Other approaches would include proper disposal of sewage, garbage, manure, and trash. Screens, bed nets, and protective clothing have become widely used and appreciated as preventive measures against flying insects. Although several other methods have been tried, they are somewhat questionable, at best. One example is the use of pet products such as flea collars. The use of flea collars for humans has not been approved by the Food and Drug Administration and may be dangerous due to the potential for pesticide absorption through the skin.

Biological Control

"Classical" biological control is based on the importation of exotic natural enemies (parasites, predators, or pathogens) and their long-term establishment in the new environment, a strategy which may then provide long-term control of the target exotic pest arthropod. Classical biological control has been actively practiced for about 100 years in the United States. Genetic manipulation of natural enemies of arthropods offers promise of enhancing their efficacy in agricultural cropping systems. A number of programs are currently being used and include (i) use of specific viruses, bacteria, protozoa, nematodes, and fungi, all of which are pathogenic for various arthropods; (ii) genetic manipulation of arthropod populations, including release of sterile males and use of insect growth regulators; and (iii) release of actual predators such as the small fish *Gambusia affinis*, which feeds on mosquito larvae. The introduction of the irradiation technique for sterilizing males of the screwworm fly, *Cochliomyia hominivorax*, has dramatically limited the development of progeny of this myiasis-producing fly throughout the southern United States. Chemosterilants are also being used, in some cases eliminating the need for radiation techniques. Insect growth regulators generally cause reproductive dysfunction or inhibit development of the adult stage. Specific vector control guidelines can be found in references 49, 80, 81.

Chemical Control

Although chemical control measures dramatically increased after World War II, the current approach involves combination methods rather than relying on chemical means alone. Concerns have been raised about a number of chemicals, particularly in terms of their possible contamination of the food chain. Also, different chemicals and approaches vary considerably throughout the world, often depending on location and financial considerations.

Most insecticides currently in use include inorganic compounds (arsenicals and fluorine compounds), botanicals (pyrethrins from pyrethrum flowers), chlorinated hydrocarbons (DDT, lindane, chlordane, dieldrin, and methoxychlor), and organophosphorus (malathion, dicapthon, and parathion) and carbamate (Baygon, Sevin, and Bendiocarb) compounds. A great deal of emphasis has been placed on the residual action of certain insecticides, compounds that remain stable on surfaces for long periods but which are effective at very low concentrations. These residual sprays are quite different from those that are sprayed ("space sprayed") for short-term effectiveness.

Unfortunately, as with all animal populations, susceptibility and resistance to these insecticides will vary; arthropods that are resistant will survive. Cross-breeding of these survivors often tends to enhance the resistance. This has occurred with insects exposed to chlorinated hydrocarbons and organophosphorus compounds.

Insecticides can be applied as sprays (immediate action or residual), aerosols (the insecticide remains suspended in air for a long time), ultra-low-volume concentrates (essentially no solvents are used), dusts (10% insecticide in talc or pyrophyllite inert carriers), granules (inorganic carrier such as vermiculite), or bait (houseflies, cockroaches, ants). Repellents and acaricides can also be used for those who are exposed to arthropods in infested areas. True repellents do not kill the arthropods but discourage them from landing or biting.

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Appendix 35.1 Medically important arthropods and their potential effect on humans

Representative species	Common name	Geographic distribution	Effect(s) on humans
Insecta			
Anoplura (sucking lice)			
Pediculus humanus humanus	Body louse	Worldwide	Skin reactions to bites; vectors of rickettsiae and spirochetes
P. humanus capitis	Head louse	Worldwide	Skin reaction to bites
Phthirus pubis	Crab louse	Worldwide	Skin reaction to bites
Mallophaga (biting lice)			
Trichodectes canis	Dog louse	Worldwide	None—can transmit *Dipylidium caninum*
Hemiptera (true bugs)			
Cimex lectularius	Bedbug	Worldwide	Skin reaction to bites
C. hemipterus	Tropical bedbug	Tropical and subtropical	Skin reaction to bites
Triatoma infestans, *Rhodnius prolixus*, *Panstrongylus megistus*	Kissing bug, conenose bug	Tropical and subtropical regions of the New World	Skin reaction to bites; vectors of *Trypanosoma cruzi*, the cause of Chagas' disease
Hymenoptera (bees, wasps, ants)			
Apis mellifera	Honeybee	Worldwide	Painful sting, potential anaphylaxis
Bombus spp.	Bumblebee	Worldwide	Painful sting, potential anaphylaxis
Various genera and species of the family Vespidae	Wasp, hornets, yellow jackets	Worldwide	Painful sting, potential anaphylaxis
Solenopsis spp.	Fire ant	Tropical America, southeastern and southwestern United States	Painful bite and multiple stings, potential anaphylaxis
Diptera (flies, mosquitoes, and their relatives)			
Culicoides spp./*Leptoconops* spp.	Biting midge	Worldwide	Serious biting pest; skin reaction to bites; vectors of several filarid nematodes
Phlebotomus spp./ *Lutzomyia* spp.	Sand fly	Worldwide/tropical region of New World	Skin reaction to bites; vectors of leishmanias, bacteria, and viruses
Simulium spp.	Blackfly, buffalo gnat	Worldwide	Serious biting pests; skin reaction to bites; vectors of *Onchocerca* and *Mansonella* spp.
Aedes spp.	Mosquito	Worldwide	Serious biting pests; skin reaction to bites; vectors of viruses, protozoa, and filaria
Anopheles spp.	Mosquito		
Culex spp.	Mosquito		
Culiseta spp.	Mosquito		
Mansonia spp.	Mosquito		
Tabanus spp.	Horsefly	Worldwide	Biting pests; painful bite followed by skin reaction
Chrysops spp.	Deerfly	Worldwide	Biting pests; vectors of tularemia and *Loa loa*
Glossina spp.	Tsetse fly	Africa	Biting pests; vectors of African trypanosomes
Siphonaptera (fleas)			
Ctenocephalides canis, *C. felis*	Dog flea, cat flea	Worldwide	None—can transmit *Dipylidium caninum*
Pulex irritans	Flea	Worldwide	Dermatitis, ulcerations
Xenopsylla cheopis	Rat flea	Worldwide	None—can transmit *Hymenolepis diminuta*
Tunga penetrans	Chigoe flea	Tropical and subtropical	Nodular swelling with subsequent ulceration

(continued)

Appendix 35.1 Medically important arthropods and their potential effect on humans *(continued)*

Representative species	Common name	Geographic distribution	Effect(s) on humans
Orthoptera (cockroaches, grasshoppers, and their relatives)			
Blatella germanica	German cockroach	Worldwide	Mechanical disseminator of pathogens; intermediate host of certain helminths
Periplaneta americana	American cockroach	Worldwide	
Various genera	Cockroach	Worldwide	
Coleoptera (beetles)			
Various genera and species	Beetles	Worldwide	Mechanical disseminators of pathogens
Various species of the family *Meloidae*	Blister beetles	Worldwide	Blistering of skin after contact with adult
Tenebrio spp.	Grain beetles	Worldwide	Intermediate hosts of tapeworms—*Hymenolepis* spp.
Lepidoptera (moths and butterflies)			
Various genera and species	Caterpillar	Worldwide	Urticating spines and hairs may cause reaction when handled or inhaled
Arachnida			
Scorpionida (scorpions)			
Various genera and species	Scorpion	Tropical and subtropical	Initially painful sting, often followed by systemic reactions
Araneidae (spiders)			
Lactrodectus mactans	Black widow spider	Americas	Bite usually painless, delayed systemic reaction
Lactrodectus spp.	Widow spider	Worldwide	Bite usually painless, delayed systemic reaction
Loxosceles reclusa	Brown recluse spider	Americas	Initial blister at wound site followed by sometimes extensive necrosis and slow healing
Loxosceles laeta	South American brown spider	South America	
Acarina (ticks and mites)			
Ornithodoros spp.	Soft tick	Worldwide	Skin reactions to bite; tick paralysis; vectors of relapsing fever
Amblyomma spp.	Hard tick	Worldwide	Skin reactions to bite; tick paralysis; vectors of rickettsias, viruses, bacteria, and protozoa
Dermacentor spp.			
Ixodes spp.			
Demodex folliculorum	Follicle mite	Worldwide	Found in sebaceous glands and hair follicles, occasional skin reactions
Sarcoptes scabiei	Human itch mite	Worldwide	Burrows in skin, causing severe itching
Trombicula spp.	Chigger, red bug	Worldwide	Intense itching at site of attachment; vectors of rickettsias
Crustacea			
Cyclops spp.	Copepod	Worldwide	Intermediate hosts of guinea worm and broadfish tapeworm
Diaptomus spp.			
Chilopoda			
Scolopendra spp.	Centipede	Tropical and subtropical	Painful bite
Pentastomida			
Linguatula serrata	Tongue worm	Tropical	Endoparasitic in internal organs

Appendix 35.2 Common arthropods: diagnostic procedures and recommended therapy

Name	Symptoms	Diagnostic procedure	Recommended therapy
Fly larvae			
Cutaneous, mucocutaneous	Painful, indolent ulcers; furuncle-like sores; dermal lesions like creeping eruption	Remove larvae, place in petri dish (blood agar plate). Identification will depend on examination of the larval stigmal plates and/or the adult fly (should often be referred to an experienced entomologist).	Local palliatives, often including antibiotics for secondary infections
Nose	Obstruction of nasal passages, facial edema, and fever		
Ear	Crawling sensations, buzzing noises, foul-smelling discharge		
Eye	Severe irritation and pain		
Intestinal	Severe nervous symptoms as well as internal irritation		Treatment with castor oil will expel these larvae
Bedbugs	Asymptomatic, edema, inflammation, indurated ring at site of feeding, asthma, anxious concern	Examination and identification of adult bug	Local palliatives and insecticides, thorough washing of all bedding
Reduviid bugs (kissing bugs, conenose bugs)	Local swelling and induration, oozing of blood from puncture site, generalized urticaria (bites commonly on exposed parts of skin, face, outer area of eye, lip)	Examination and identification of adult bug	Local palliatives
Fleas			
Tunga penetrans	Adult female burrows into skin (often toes and feet), causing nodular swelling, subsequent ulceration and festering sores	Examination and identification of adult flea	Wearing shoes, flea removal aseptically with needle, bathing of wound and treating with antiseptic
Pulex irritans (human), *Xenopsylla cheopis* (rat), *Ctenocephalides canis* (dog)	Asymptomatic; roseate raised lesion, frequently edematous, indurated, or pustular (bites are more common on lower extremities)		Local palliatives, use of recommended insecticides
Lice			
Pediculus spp., *Phthirus pubis*	Cutaneous lesions, roseate elevated papule, intense pruritus, lesions may become secondarily infected by bacteria	Examination and identification of adult and/or eggs on hair shaft	Shampoo or ointment (1% lindane); dusts with 1% malathion; bedding can be fumigated with ethyl formate; remaining nits can be removed with fine-toothed comb
Ticks			
Tick paralysis	Generalized toxemia, fever (104°F), rapid ascending flaccid paralysis, difficulty in swallowing and respiration, and death; little or no change in white cell count	Careful examination of body, particularly back of the neck and along spinal column	Prompt removal of the engorging female tick will usually limit progression of paralysis (due to toxin in tick saliva)
Other ticks and bites	Inflammatory infiltration of tissues, local hyperemia, edema, hemorrhage (histolysis at the bite site as a result of salivary secretions)	Examination and identification of adult tick	Anterior portion should be grasped with forceps close to the skin and pulled *straight out*; leaving mouth parts may cause severe tissue reactions and possible secondary infections

(continued)

Appendix 35.2 Common arthropods: diagnostic procedures and recommended therapy *(continued)*

Representative species	Common name	Geographic distribution	Effect(s) on humans
Mites			
Sarcoptes scabiei (itch mite)	Small skin vesicles; scratching results in bleeding and scab formation followed by secondary infection	Skin scraping (avoid excoriated lesions); get material from early papule or burrow (between fingers, wrists, elbows, axillae, penis, scrotum, buttocks, backs of knees, ankles, toes, under breasts)	Topical application of 1% lindane in lotion or ointment (may require one to three applications); should be applied after scrubbing and soaking in warm water to expose lesions
Spiders			
Black widow spider	Symptoms will depend on amount of venom injected; may include abdominal cramps, hypertension, and convulsions	History and/or identification of spider	Specific antivenom is available (patients should be tested for horse serum sensitivity before use); other supportive therapy depending on symptoms
Violin spider, brown recluse spider	Bite may be painless; mild to severe pain within hours; erythema, vesicle formation, and itching followed by chills, headache, and nausea; the lesion becomes necrotic, spreads, and heals very slowly; kidney damage and death may also occur	Identification of spider	Prompt administration of steroids, early surgical excision of lesion (skin grafting may be necessary), treatment of secondary infections

Appendix 35.3 Common terms used in discussing arthropods

Term	Definition
Antennae	Segmented appendages located in the lateral side of the head
Capitulum	"False head" found in arachnids; basal portion of structure is "basis capituli"
Cell	Area of wing enclosed by veins
Cerci	Appendages (slender, two) at terminal abdominal segment
Chelicerae	Appendages (grasping or cutting) in place of jaws; found in arachnids
Claw	Appendage (sharp, curved) at end of leg
Complete metamorphosis.	Cycle of development (e.g., egg, larva, pupa, adult); each stage distinct from others
Compound eye	Visual elements (large number) grouped on each side of head
Costa	Thickening of the anterior edge of the wing
Coxa	Proximal segment of the leg
Ecdysis	Shedding of larval skin during transformation from one stage to another
Emergence	Appearance of winged insect from the pupal stage
Epipharynx	Organ attached to inner part of labrum
Exoskeleton	External skeleton
Fat body	Group of cells (widely distributed, food store); contains protein, fats, glycogen
Festoons	Notches seen at posterior part of body of hard ticks
Gill	Specialized respiratory organ seen in aquatic insects
Haltere	Club-shaped structure in place of second pair of wings in Diptera
Hypopharynx	Tongue-shaped structure; lies between labrum and labium; connected to salivary duct
Hypostome	Lower lip which takes the form of an elongated structure armed with teeth or hooks in ticks
Incomplete metamorphosis	Morphology between adults and immature stages similar; stages resemble each other
Labial palpi	Pair of appendages arising from each side of the labrum
Labium	Lower lip which forms floor of mouth
Labrum	Upper lip which forms roof of the mouth
Longitudinal veins	Series of six veins following the subcosta; numbered in sequence
Maggot	Common name given to larva of some Diptera (flies)
Mandibles	Pair of upper jaws
Maxillae	Pair of lower jaws
Mesonotum	Dorsal surface of second thoracic segment
Myiasis	Infestation of human and animal tissues with fly maggots
Nymph	Immature stage in insects undergoing incomplete metamorphosis
Ocellus	Simple eye
Ovipositor	Tubular structure through which eggs are laid
Palmate hairs	Fan-shaped hairs on dorsal surface of anopheline larva
Pedipalp	Second pair of appendages which arise from cephalothorax in arachnids
Pleuron	Lateral part of a segment
Pronotal comb	Anterior segment of thorax
Pronotum	Dorsal surface of first thoracic segment
Prothorax	Anterior segment of thorax
Pseudotracheae	Small tubes found on labella (through which fluid is sucked) in houseflies and members of the *Tabanidae*
Pygidium	Pincushion-like structure seen on ninth segment of fleas
Scutellum	Small posterior section of the tergum
Scutum	Chitinous shield seen on the dorsum of Acarina
Sensilla	Hairs which have sensory function
Setae	Hairlike structures which are hollow internally
Spermatheca	Sperm-storing organ in lower genital tract of females
Spiracles	Respiratory openings
Subcosta	Vein lying posterior to the costa
Tergum	Dorsal plate on thoracic segment
Tissue invasion	Infestation (superficial tissues); infection (deeper tissues)
Tracheae	Ringed breathing tubes
Vector	Transmitter of infection (arthropods)

Appendix 35.4 Dichotomous key to some of the arthropods important in human disease and/or disease transmission

A.	Legs:	Three or four pairs	B
	Legs:	Five or more pairs	V
B.	Legs:	Three pairs; antennae present	C
	Legs:	Four pairs; antennae absent	L
C.	Wings:	Well developed	D
	Wings:	Absent or rudimentary	L
D.	Wings:	One pair (**Diptera**: flies, mosquitoes, midges)	E
	Wings:	Two pairs	F
E.	Wings:	Scales present (**Diptera**: mosquitoes)	
	Wings:	Scales absent (**Diptera**: flies)	
F.	Mouth parts:	Sucking (long proboscis present)	G
	Mouth parts:	Chewing (long proboscis absent)	H
G.	Wings:	Scales present; proboscis coiled (**Lepidoptera**: butterflies, moths)	
	Wings:	Scales absent; proboscis not coiled (**Hemiptera**: bedbugs, kissing bugs)	
H.	Wings:	Both pairs membranous, size may vary	I
	Wings:	Front pair leathery or shell-like, covers second pair	J
I.	Wings:	Both pairs similar in size (**Isoptera**: termites)	
	Wings:	Hind wing smaller than front wing (**Hymenoptera**: bees, hornets, wasps)	
J.	Wings:	No distinct veins; front wings horny or leathery; meet as straight line in middle	K
	Wings:	Distinct veins; front wings horny or leathery; overlap in middle (**Blattaria**: cockroaches)	
K.	Abdomen:	Prominent cerci or forceps, longer than wings (**Dermaptera**: earwigs)	
	Abdomen:	No prominent cerci or forceps, covered by wings (**Coleoptera**: beetles)	
L.	Abdomen:	Three long terminal tails (**Thysanura**: silverfish and firebrats)	
	Abdomen:	No terminal tails	M
M.	Abdomen:	Narrow waist (**Hymenoptera**: ants)	
	Abdomen:	No narrow waist	N
N.	Abdomen:	Prominent pair of cerci or forceps (**Dermaptera**: earwigs) (see also K)	
	Abdomen:	No cerci or forceps	O
O.	Body:	Flattened laterally; antennae folded into head grooves (**Siphonaptera**: fleas)	
	Body:	Flattened dorsoventrally; antennae project from side of head	P
P.	Antennae:	Nine or more segments	Q
	Antennae:	Three to five segments	R
Q.	Pronotum:	Covers head (**Blattaria**: cockroaches) (see also J)	
	Pronotum:	Does not cover head (**Isoptera**: termites) (see also I)	
R.	Mouth parts:	Tubular jointed beak; three- to five-segmented tarsi (**Hemiptera**: bedbugs)	
	Mouth parts:	Retracted into head or chewing type; one- or two-segmented tarsi	S
S.	Mouth parts:	Retracted into head, adapted for sucking blood (**Anoplura**: sucking lice)	
	Mouth parts:	Chewing type (**Mallophaga**: chewing lice)	

(continued)

Appendix 35.4 *(continued)*

T.	Body:	Oval, consisting of a single saclike region (**Acari**: ticks and mites)	
	Body:	Divided into two distinct regions, cephalothorax and abdomen	U
U.	Abdomen:	Joined to cephalothorax by slender waist (**Araneae**: spiders)	
	Abdomen:	Joined broadly to cephalothorax, stinger present (**Scorpiones**: scorpions)	
V.	Legs, swimmerets:	Five to nine pairs of legs or swimmerets; one or two pairs of antennae (**Crustacea**: copepods, crabs, crayfish)	
	Legs, swimmerets:	Ten or more pairs of legs; swimmerets absent, one pair of antennae	W
W.	Legs per body segment:	One pair (**Chilopoda**: centipedes)	
	Legs per body segment:	Two pairs (**Diplopoda**: millipedes)	

[a] Adapted from references 1–9, 55.

Appendix 35.5 Key to the important myiasis-producing larvae[a]

1.	Body with spinous or fleshy processes laterally and dorsally or terminally	*Fannia*
	Body smooth or with short spines, but *never* having long fleshy lateral processes	2
2.	Body with a long slender tail or caudal process capable of some extension and retraction	*Eristalis*
	Body sometimes narrowed posteriorly, but *never* with a long flexible caudal process capable of some extension and retraction	3
3.	Larvae more or less grub-like; most species slightly flattened dorsoventrally	4
	Larvae maggot-like, or typical "muscoid" shape, tapering anteriorly, broadly truncate at the posterior end; cross section more or less circular at all points	5
4.	Posterior spiracular plate with three distinct slits	*Dermatobia*
	Posterior spiracular plate with many fine openings	*Hypoderma*
5.	Posterior spiracles within a well-chitinized and complete ring encircling the button area; spiracles *never* in a distinct depression	6
	Posterior spiracles with the button very slightly chitinized or absent; chitinized ring incomplete; spiracles in a distinct depression or flush with surface	8
6.	Button area with spiracular slits nearly straight	7
	Button area with spiracular slits sinuous, with at least a double curve	9
7.	Principal transverse subdivisions of spiracular slits well marked, usually not more than six; both ring and button heavily chitinized, the ring thickened into points at two places between the slits	*Calliphora*
	Transverse subdivisions of spiracular slits less distinctly marked, from 6 to 20 in number, ring and button less heavily chitinized, the ring thickened into point at only one place between the slits	*Phaenicia*
8.	Posterior spiracles in a more or less distinct pit or depression, vestigial button usually present; integument rather smooth	*Sarcophaga*
	Posterior spiracles flush with surface; integument rather spiny (Western Hemisphere)	*Cochliomyia*
9.	Posterior spiracular plates D-shaped, each slit thrown into several loops	*Musca*
	Posterior spiracular plates triangular, with rounded corners; spiracular slits S-shaped; button indistinct, centrally placed	*Stomoxys*

[a] Adapted from Prendergast BF (ed), *Filth Flies, Technical Guide 30*, Armed Forces Pest Management Board, Washington, DC, 2002 (reviewed and validated 2014).

36

Treatment of Parasitic Infections

Chemotherapy plays a very important role not only in reducing patient morbidity and mortality but also in reducing transmission of the parasitic infection. Many of the drugs used to treat parasitic infections have serious side effects; therefore, before initiation of therapy, it is important to consider the following factors: health of the patient, parasite drug resistance, accuracy of the original dose, potential drug toxicity, and the need for follow-up examinations to monitor therapy. The mechanisms of action of most antiparasitic drugs are not well known, including those involving potential drug toxicity to the patient. Because of the limited resources of developing nations, where a majority of parasitic infections occur, there is little commercial incentive for developing effective therapeutics or vaccines. For some drugs, such as triclabendazole, used extensively in veterinary practices, less is known about adverse side effects or toxicity to humans. Specific information can be obtained from the Centers for Disease Control and Prevention, CDC Drug Service, Atlanta, GA (day, 404-639-3670; evenings, weekends, and holidays, 404-639-2888). One can also obtain drugs from CDC that are not commercially available in the United States.

Tables 36.1 and 36.2 provide information on the most commonly used drugs for treating parasitic infections; not all drugs are included, and some drugs listed are not available in the United States. In Table 36.1, unless specifically provided, pediatric doses are the same as that given for adults. Also, throughout the total literature, there may be some variations among the drug of choice and alternative options for the various parasitic infections listed in Table 36.1. References 1 to 3 contain overall drug information, while references 4 to 51 are very recent and cover almost all human parasitic infections. As an example, they begin with free-living amebae and end with trypanosomiasis.

Albendazole (Albenza) (Amedra)

Preparation

The drug is available as 200-mg tablets and is used for the treatment of *Ancylostoma caninum* (eosinophilic enterocolitis), anisakiasis, ascariasis, baylisascariasis, capillariasis, clonorchiasis, cutaneous larva migrans, hookworm, neurocysticercosis and hydatid disease, enterobiasis, loiasis, microsporidiosis, opisthorchiasis, strongyloidiasis, visceral larva migrans, and trichuriasis.

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Administration

The drug is given with meals. It is poorly absorbed from the gastrointestinal tract due to its low aqueous solubility. Concentrations in plasma are negligible or undetectable because it is rapidly converted to the sulfoxide metabolite prior to reaching the systemic circulation. The systemic anthelmintic activity has been attributed to the primary metabolite, albendazole sulfoxide. Oral bioavailability appears to be enhanced when albendazole is coadministered with a fatty meal (estimated fat content, 40 g) as evidenced by higher (up to 5-fold on average) concentrations of albendazole sulfoxide in plasma compared to the concentration in plasma in fasted state.

Toxicity

Side effects include headache, vomiting, diarrhea, abdominal pain, increase in serum glutamic-oxaloacetic transaminase levels, and fever; migration of *Ascaris* through the mouth and nose may be seen.

Rare fatalities associated with the use of albendazole, due to granulocytopenia or pancytopenia, have been reported. Blood counts should be monitored at the beginning of each 28-day cycle of therapy and every 2 weeks during therapy with albendazole. Albendazole therapy may be continued if the total white blood cell count and absolute neutrophil count decrease appear modest and do not progress. Albendazole should not be used in pregnant women except in clinical circumstances where no alternative management is appropriate. Patients should not become pregnant for at least 1 month following cessation of albendazole therapy. If a patient becomes pregnant while taking this drug, the therapy should be discontinued immediately; the patient should be apprised of the potential hazard to the fetus.

Patients being treated for neurocysticercosis should receive appropriate steroid and anticonvulsant therapy as required. Oral or intravenous corticosteroids should be considered to prevent cerebral hypertensive episodes during the first week of anticysticercal therapy. Cysticercosis may, in rare cases, involve the retina. Before initiation of therapy for neurocysticercosis, the patient should be examined for the presence of retinal lesions. If such lesions are visualized, the need for anticysticercal therapy should be weighed against the possibility of retinal damage caused by albendazole-induced changes to the retinal lesion.

Contraindications

Due to lack of safety information, albendazole should not be given during pregnancy or lactation. Blood counts and liver function tests should be performed at the beginning of each 28-day treatment cycle and every 2 weeks.

Amphotericin B (AmBisome) (Gilead)

Preparation

The drug is supplied in vials containing 50 mg of amphotericin B inserted into a liposomal membrane and is used to treat mucocutaneous and visceral leishmaniasis and primary amebic meningoencephalitis. The product is lyophilized, and following reconstitution with sterile water, the pH is 5.0 to 6.0. It should be stored in the refrigerator and protected from light. It may be reconstituted with the addition of 12 ml of sterile water for injection USP, without a bacteriostatic agent, to make a 4-mg/ml solution. This may be further diluted by adding 1 ml of the 4-mg/ml solution to 40 ml of 5% glucose solution to make a final concentration of 0.1 mg of amphotericin per ml. The reconstituted product concentrate may be stored for up to 24 h under refrigeration. Injection of AmBisome should begin within 6 h of dilution with 5% dextrose injection. An existing intravenous line must be flushed with 5% dextrose injection prior to infusion of AmBisome. If this is not feasible, AmBisome should be administered through a separate line. AmBisome should never be reconstituted with saline, and saline must not be added to the reconstituted mixture. The mixture must not be mixed with any other drugs; this may cause precipitation of AmBisome.

Administration

AmBisome should be administered intravenously (i.v.), using a controlled infusion device, over a period of approximately 120 min. The infusion time may be reduced to approximately 60 min in patients in whom the treatment is well tolerated.

Toxicity

AmBisome-treated pediatric patients had a lower incidence of infusion-related fever, chills or rigors, and vomiting on day 1 than did amphotericin B deoxycholate-treated patients. The incidence of infusion-related cardiorespiratory events was also lower when AmBisome was used. In adult patients, AmBisome treatment resulted in a lower incidence of chills, hypertension, hypotension, tachycardia, hypoxia, hypokalemia, and various events related to decreased kidney function compared with amphotericin B deoxycholate.

Contraindications

AmBisome is contraindicated in patients who have demonstrated or have known hypersensitivity to amphotericin B deoxycholate or any other constituents of the product unless, in the opinion of the treating physician, the benefit of therapy outweighs the risk.

(continued on p. 1153)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a]

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
Acanthamoeba spp. Granulomatous amebic encephalitis	Pentamidine Sulfadiazine Flucytosine	4 mg/kg i.v. once/day 1.5 g every 6 h PO 37.5 mg/kg PO every 6 h (max 150 mg/kg/day)	Monitor clinical course; usually susceptible *in vitro* to pentamidine; addition of pentamidine based on clinical judgment
	Fluconazole	12 mg/kg i.v. once/day	Consult with CDC
	Miltefosine	Available from CDC	
Keratitis	Polyhexamethylene biguanide (Baquacil) (PHMB) or 0.2% chlorhexidine	0.02% applied topically hourly or every half hour for 3 days, then every 4 h	Monitor clinical course; combination therapy highly recommended; PHMB available at Leiter's Park Ave. Pharmacy, San Jose, CA (800-292-6773) (www.leiterrx.com) Prolonged therapy (≥6 mo) may be necessary
	plus/or		
	Propamidine isethionate (Brolene)	0.1% applied topically	
	plus		
	Neomycin-polymyxin B-gramicidin	Ophthalmic solution	Since most cysts are resistant to neomycin, this approach is no longer recommended.
Ancylostoma caninum (eosinophilic enterocolitis)	Drugs of choice: Albendazole	400 mg PO once	Monitor clinical course; endoscopic removal an option
	or		
	Mebendazole	100 mg PO BID × 3 days or 500 mg once	
Ancylostoma duodenale *Necator americanus* (hookworm)	Drugs of choice: Albendazole	400 mg PO once	Fecal examinations 2–4 wk posttherapy
	or		
	Mebendazole	100 mg PO BID × 3 days or 500 mg once	
	or		
	Pyrantel pamoate	11 mg/kg (max 1 g) × 3 days	If eggs are rare in fecal concentrations, there is no need to repeat therapy
Angiostrongylus cantonensis	Mebendazole	100 mg BID × 5 days	Monitor clinical course; may want to add corticosteroid; shorten course of infection
Angiostrongylus costaricensis	Drug of choice: Mebendazole	[pa200–400 mg TID × 10 days	Monitor clinical course; may want to add corticosteroid; shorten course of infection
	Alternative: Thiabendazole	75 mg/kg/day in 3 doses × 3 days (max 3 g/day)	
Anisakis spp.	Surgical or endoscopic removal Albendazole	400 mg PO BID × 3–5 days	Monitor clinical course; albendazole has also been reported to be successful

Ascaris lumbricoides (roundworm)	Drugs of choice:		
	Albendazole	400 mg PO once	Fecal examinations 2–4 wk posttherapy; nitazoxanide may be effective against a number of protozoa and helminths; available as 500-mg tablets and oral suspension—take with food
	or		
	Mebendazole	100 mg BID × 3 days or 500 mg once	
	or		
	Ivermectin	150–200 μg/kg PO once	
Babesia spp.	Drugs of choice:		
	Atovaquone plus	750 mg PO BID × 7–10 days Children: 40 mg/kg/day PO in 2 doses × 7–10 days	Monitor clinical course; repeat blood films if symptoms persist; concurrent babesiosis, Lyme disease and/or human granulocytic anaplasmosis may occur; atovaquone available in oral suspension—take with meal to increase absorption
	Azithromycin	500–1,000 mg PO on day 1, then 250–500 mg PO on days 2–10 Children: 10 mg/kg (max 500 mg/dose) PO on day 1, then 5 mg/kg/day (max 250 mg dose) PO on days 2–10	
	or		
	Clindamycin	300–600 mg i.v. QID or 600 mg PO TID × 7–10 days Children: 20–40 mg/kg/day (max 600 mg/dose) i.v. or PO in 3 to 4 doses × 7–10 days	Take with full glass of water to minimize esophageal ulceration
	plus		
	Quinine	650 mg PO TID or QID × 7–10 days Children: 24 mg/kg/day (max 600 mg/dose) PO in 3 doses × 7–10 days	
Balamuthia mandrillaris (granulomatous amebic encephalitis)	Azithromycin	500 mg i.v. once/day Children: 20 mg/kg i.v. once/day (max 500 mg/day)	Monitor clinical response and organisms in CSF; direct wet mounts of CSF and cultures Pentamidine can also be added based on clinical judgment
	Alternatives:		
	Clarithromycin (Biaxin)	14 mg/kg/day PO in 2 doses (max 2 g/day)	These 4 drugs used in combination
	Pentamidine	4 mg/kg i.v. once/day	
	Sulfadiazine	1.5 g PO every 6 h Children: 200 mg/kg/day PO in 4–6 doses (max 6 g/day)	
	Flucytosine (Ancobon)	37.5 mg/kg PO every 6 h (max 150 mg/kg/day)	
	Fluconazole	12 mg/kg i.v. once/day	
Balantidium coli	Drug of choice: Tetracycline	500 mg PO QID × 10 days Children (≥8 yr): 40 mg/kg/day in 4 doses × 10 days (max 2 g/day) PO	At least 3 negative fecal examinations 1 mo after therapy; contraindicated in pregnancy and in children <8 yr; take 1 h before or 2 h after meals and/or dairy products

(continued)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a] *(continued)*

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
	Alternatives:		
	Iodoquinol	650 mg PO TID × 20 days Children: 40 mg/kg/day (max 2 g/day) in 3 doses × 20 days	
	or		
	Metronidazole	500–750 mg PO TID × 5 days Children: 35–50 mg/kg/day PO in 3 doses × 5 days	
Baylisascaris procyonis (severe visceral and neural larva migrans) (raccoon roundworm)	Drugs of choice: Albendazole (mebendazole, thiabendazole, levamisole, ivermectin can be used if albendazole not available)	Check with CDC Drug Service Therapy must be started as soon as possible (up to 3 days after possible infection) Albendazole 37 mg/kg/day PO × 20 days combined with high-dose steroids	Steroids may also be helpful in eye and CNS infections; laser photocoagulation therapy has been used to destroy larvae in the retina; albendazole plus steroids recommended for children with known exposure
Blastocystis spp. (may comprise multiple strains, some pathogenic and some nonpathogenic)	Metronidazole	750 mg PO TID × 10 days Children: 35–50 mg/kg/day in 3 doses × 10 days	May see resistance with metronidazole; also may be reinfection; most common protozoan found in GI tract throughout the world (more common than *Giardia* or *Dientamoeba*)
	Trimethoprim/sulfamethoxazole	1 DS tab PO BID × 7 days	
	Iodoquinol	650 mg PO TID × 20 days Children: 30–40 mg/kg/day in 3 doses × 20 days	Fecal examinations 2 wk to 1 mo after therapy (must include permanent stain); metronidazole resistance may be common; another alternative would be TMP/SMX or nitazoxanide in children
Brugia malayi *Brugia timori*	Drug of choice: Diethylcarbamazine (available from CDC)	6 mg/kg/day PO in 3 doses × 1 or 12 days	Blood smears, Knott concentration, and membrane filtration of blood for microfilariae 2–4 wk after treatment; for patients with no microfilariae in the blood, full doses can be given from day 1 (6 mg/kg/day PO in 3 doses × 1 or 12 days)
Capillaria philippinensis	Drug of choice: Mebendazole	200 mg PO BID × 20 days	Multiple fecal examinations for eggs several months after completion of therapy
	Alternative: Albendazole	400 mg PO BID × 10 days	
Clonorchis (*Opisthorchis*) *sinensis* (Chinese liver fluke)	Drugs of choice: Praziquantel	75 mg/kg/day PO in 3 doses × 1–2 days	Fecal examinations for eggs 1 and 3 mo after therapy
	or Albendazole	10 mg/kg × 7 days	
Cryptosporidium spp.	Drugs of choice: Nitazoxanide	500 mg PO BID × 3 days Children 1–3 yr: 100 mg PO BID × 3 days 4–11 yr: 200 mg PO BID × 3 days >12 yr: 500 mg PO BID × 3 days	Take with food
	or Paromomycin	25–35 mg/kg/day in 2 or 4 doses	Monitor clinical response and perform stool examinations during and after experimental therapy (acid-fast stain or fecal immunoassay); may be combined with azithromycin (600 mg/day)

Cutaneous larva migrans (creeping eruption, dog and cat hookworm) (*Ancylostoma braziliense*, *Ancylostoma caninum*)	Drugs of choice:		
	Albendazole	400 mg PO daily × 3 days	Monitor clinical response to therapy
	or		
	Ivermectin	200 μg/kg PO daily × 1–2 days	
Cyclospora cayetanensis	TMP-SMX	TMP 160 mg, SMX 800 mg (1 DS tab) PO BID × 7–10 days Children: TMP 10 mg/kg, SMX 50 mg/kg/day PO BID × 7–10 days	Monitor clinical course and perform stool examination using modified acid-fast stains or autofluorescence
	Alternative: Ciprofloxacin	500 mg PO BID × 7 days	
Cysticercosis (*Taenia solium*)	Albendazole	15 mg/kg/day (max 800 mg) PO in 2 doses × 8–30 days; can be repeated if necessary	Monitor clinical response to therapy; follow up with CT scans; corticosteroids should be given 2–3 days before and during therapy; damage may occur when treating ocular or spinal cysts, even using corticosteroids; antiseizure medication may also be required; take with food
	or		
	Praziquantel	50 mg/kg/day PO × 15 days	
	or		
	Surgery		
Cystoisospora belli	TMP-SMX	160 mg of TMP, 800 mg of SMX (1 DS tab) PO BID × 10 days Children: TMP 10 mg/kg/day, SMX 50 mg/kg /day PO BID × 10 days	Fecal examinations 1–2 wk after therapy; some areas of endemicity within South America; can be seen in immunocompromised patients
Dientamoeba fragilis	Drugs of choice:		
	Paromomycin	25–35 mg/kg/day PO in 3 doses × 7 days	Fecal examinations 2–4 wk after therapy (must include permanent stained smear); take with meal
	or		
	Iodoquinol	650 mg TID × 20 days Children: 30–40 mg/kg/day (max 2 g) in 3 doses × 20 days	Take after meals
	or		
	Metronidazole	500–750 mg PO TID × 10 days Children: 35–50 mg/kg/day PO in 3 doses × 10 days	In one study, single-dose ornidazole (available in Europe) was effective and better tolerated than metronidazole
Diphyllobothrium latum (broad fish tapeworm)	Drug of choice: Praziquantel	5–10 mg/kg once	Fecal examinations for eggs and proglottids 1 and 3 mo after therapy; take with liquids during a meal
	Alternative: Niclosamide	2 g once Children: 50 mg/kg once	Chew thoroughly or crush, swallow with water

(continued)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a] *(continued)*

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
Dipylidium caninum (dog and cat tapeworm)	Drug of choice: Praziquantel	5–10 mg/kg once	Fecal examinations for eggs and proglottids 1 and 3 mo after therapy
	Alternative: Niclosamide	2 g once Children: 50 mg/kg once	
Dracunculus medinensis (guinea worm)	Metronidazole	250 mg TID × 10 days Children: 25 mg/kg/day PO (max 750 mg/day) in 3 doses × 10 days	Monitor clinical course; slow extraction of worm; drugs not curative, but decrease inflammation; wound care and pain management may be sufficient; treat entire household
Echinococcus granulosus (hydatid cyst)	Drugs of choice:		
	Albendazole	15 mg/kg/day (max 800 mg) PO in 2 doses × 1–6 mo	Monitor clinical response to therapy; follow up with CT, ultrasonography, and radionucleotide scans; take with food
	or		
	Surgery		Praziquantel can be used preoperatively or in case of spillage of cyst contents during surgery
Echinococcus multilocularis (alveolar hydatid cyst)	Surgery	If surgery is unsuccessful or contraindicated, albendazole may be used	Monitor clinical response to therapy; follow up with CT, ultrasonography, and radionucleotide scans
	or		
	Albendazole	15 mg/kg/day (max 800 mg) PO in 2 doses ≥2 yrs	Long-term therapy can stabilize but rarely cure infection
Entamoeba histolytica	Drugs of choice:		
	Iodoquinol; taken after meals	650 mg/kg PO TID × 20 days Children: 30–40 mg/kg/day in 3 doses × 20 days (max 2 g)	Fecal examinations 2 wk to 1 mo after therapy (must include permanent stained smear); EIAs for confirmation of true *E. histolytica* are available; trophozoites containing ingested RBCs can be identified as *E. histolytica*
	or		
	Paromomycin; with meal	25–35 mg/kg/day PO in 3 doses × 7 days	
	or		
	Diloxanide furoate (alternative)	500 mg PO TID × 10 days Children: 20 mg/kg/day in 3 doses × 10 days	Not available commercially
Mild to moderate intestinal disease	Metronidazole	500–750 mg PO TID × 7–10 days Children: 35–50 mg/kg/day PO in 3 doses × 7–10 days	Fecal examinations 2 wk to 1 mo after therapy (must include permanent stained smear); EIAs for confirmation of true *E. histolytica* are available; follow up with course of iodoquinol or paromomycin used to treat asymptomatic amebiasis
	or		
	Tinidazole	2 g/day PO × 3 days Children: ≥3 yrs, 50 mg/kg (max 2 g) PO in 1 dose ≥3 days	Tablets can be crushed and mixed with cherry syrup

	Either followed by:		
	Iodoquinol	650 mg PO TID × 20 days Children: 30–40 mg/kg/day (max 2 g) PO in 3 doses × 20 days	
	or		
	Paromomycin	25–35 mg/kg/day PO in 3 doses × 7 days	
Invasive amebiasis	Metronidazole	750 mg PO (or i.v.) TID × 7–10 days Children: 35–50 mg/kg/day PO (or i.v.) in 3 doses × 7–10 days	Monitor clinical response to therapy; follow up with CT, ultrasonography, and radionucleotide scans
	or		
	Tinidazole	2 g once daily PO × 5 days Children: ≥3 yrs, 50 mg/kg/day (max 2 g) PO every day × 5 days	Tablets can be crushed and mixed with cherry syrup
	Either followed by:		
	Iodoquinol	650 mg PO TID × 20 days Children: 30–40 mg/kg/day (max 2 g) PO in 3 doses × 20 days	
	or		
	Paromomycin	25–35 mg/kg/day PO in 3 doses × 7 days	
Entamoeba polecki	Metronidazole	750 mg TID × 10 days Children: 35–50 mg/kg/day in 3 doses × 10 days	Fecal examinations 2 wk to 1 mo after therapy (must include permanent stained smear)
Enterobius vermicularis (pinworm)	Drugs of choice:		Monitor clinical response; follow up with Scotch tape check of perianal area if symptoms persist (may take 4–6 consecutive negative tapes to rule out infection)
	Albendazole	400 mg PO once; repeat after 2 wk	
	or		
	Mebendazole	100 mg once, repeat after 2 wk	
	or		
	Pyrantel pamoate	11 mg/kg base PO once (max 1 g); repeat in 2 wks	
Fasciola hepatica (sheep liver fluke)	Drug of choice: Triclabendazole	10 mg/kg PO once or twice	Fecal examinations for eggs 1 mo after therapy; problems with availability of triclabendazole
	Alternatives:		
	Bithionol	30–50 mg/kg PO on alternate days × 10–15 doses	
	or		
	Nitazoxanide	500 mg PO BID × 7 days Children: 1–3 yrs: 100 mg PO bid × 7 days 4–11 yrs: 200 mg PO bid × 7 days >12 yrs: 500 mg PO bid × 7 days	
Fasciolopsis buski (giant intestinal fluke)	Drug of choice: Praziquantel	75 mg/kg/day PO in 3 doses × 1 day	Fecal examinations for eggs 1 mo after therapy

(continued)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a] *(continued)*

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
Giardia lamblia (*G. duodenalis*, *G. intestinalis*)	Drugs of choice:		
	Tinidazole (availability an issue)	2 g PO once Children: ≥3 yrs: 50 mg/kg PO once	Fecal examinations 2 wk to 1 mo posttherapy (must include permanent stained smear or immunoassay); Entero-Test if stools are negative and patient is symptomatic; immunoassays for stool antigen available
	or		
	Metronidazole	250 mg PO TID × 5–7 days Children: 15 mg/kg/day PO in 3 doses × 5–7 days	Fecal examinations 2 wk to 1 mo posttherapy (must include permanent stained smear or immunoassay); Entero-Test if stools are negative and patient is symptomatic; immunoassays for stool antigen available
	or		
	Nitazoxanide	500 mg PO BID × 3 days Children: 1–3 yr: 100 mg PO BID × 3 days 4–11 yr: 200 mg PO BID × 3 days >12 yrs: 500 mg PO BID × 3 days	
	Alternatives:		
	Paromomycin	25–35 mg/kg/day PO in 3 doses × 5–10 days	
	or		
	Furazolidone	100 mg PO QID × 7–10 days Children: 6 mg/kg/day PO in 4 doses × 7–10 days	
	or		
	Quinacrine	100 mg PO TID × 5 days Children: 6 mg/kg/day PO in 3 doses × 5 days (300 mg/day)	Not available commercially
Gnathostoma spinigerum	Surgical removal		Check for additional migratory swellings. Treat all patients with medication whether surgery is attempted or not.
	plus		
	Albendazole	400 mg PO BID × 21 days	
	or		
	Ivermectin	200 µg/kg/day PO × 2 days	
Gongylonema spp.	Surgical removal		Check buccal cavity and esophagus
	plus		
	Albendazole	400 mg PO BID × 3 days	
Heterophyes heterophyes (intestinal fluke)	Praziquantel	75 mg/kg/day PO in 3 doses × 3 days	Fecal examinations for eggs 1 mo after therapy

Hymenolepis nana (dwarf tapeworm)	Drug of choice: Praziquantel	25 mg/kg PO once	Fecal examinations for eggs and proglottids 2 wk and 3 mo after therapy
	Alternative: Niclosamide	2 g PO daily × 7 days Children: 11–34 kg: 1 g PO on day 1, then 500 mg/day PO for 6 days >34 kg: 1.5 g PO on day 1, then 1 g/day PO × 6 days	Must be thoroughly chewed or crushed and swallowed with water
Leishmania spp.	Drugs of choice:		
Cutaneous Mucocutaneous Visceral	Stibogluconate sodium for cutaneous, mucocutaneous; 2nd choice for visceral	20 mg of antimony/kg/day i.v. or i.m. × 20 days	For *L. donovani*, monitor clinical response to therapy; for other *Leishmania* spp., perform smear and culture of lesions 1–2 wk after therapy
	or		
	Meglumine antimonite: 2nd choice for cutaneous and mucocutaneous; 3rd choice for visceral	20 mg of antimony/kg/day i.v. or i.m. × 20 days	
	or		
	Amphotericin B: mucocutaneous	0.5–1.0 mg/kg/day by slow infusion daily or every 2 days for up to 4–8 wk	
	or		
	Liposomal amphotericin B: drug of choice for visceral	18–21 mg/kg over 5 days 3 mg/kg/day i.v. on days 1–5, 14, and 21 (FDA approved) Children: 2 doses of 10 mg/kg each	Immunocompromised: 4 mg/kg on days 1–5, 10, 17, 24, 31, and 38. The total dosage is more important than the number of infusions or duration of therapy.
	or		
	Miltefosine: alternate drug for all three types	2.5 mg/kg/day PO (max 150 mg/day) × 28 days	
	Alternatives :		
	Pentamidine (cutaneous)	2–3 mg/kg daily or every 2 days i.v. or i.m. for 4–7 doses	
	or		
	Paromomycin (cutaneous)	Topically twice daily × 10–20 days	
Lice infestation (*Pediculus humanus*, *Pediculus capitis*, *Phthirus pubis*)	Pyrethrins with piperonyl butoxide	Topically, 2 × at least 7 days apart	Eyelashes, use petrolatum ointment 2–4 × /day × 8–10 days; pubic lice, use 1% permethrin, pyrethrins with piperonyl butoxide, or ivermectin
	or		
	0.5% Ivermectin lotion	Topically once	
	or		
	0.9% Spinosad susp	Topically, 2 × at least 7 days apart	
	or		
	1% Permethrin	Topically, 2 × at least 7 days apart	
	or		

(continued)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a] *(continued)*

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
	5% Benzyl alcohol lotion	Topically, 2 × at least 7 days apart	
	or		
	0.5% Malathion	Topically 2 × at least 7 days apart	
	Alternative: Ivermectin	200 or 400 µg/kg PO	Safety in young children and pregnant women not established
Loa loa (African eye worm)	Drug of choice: Diethylcarbamazine (available from CDC) In heavy infections with *Loa loa*, rapid killing of microfilariae can provoke encephalopathy	9 mg/kg /day in 3 doses × 21 days 300 mg once/wk recommended for prevention	Blood smears, Knott concentration, and membrane filtration of blood for microfilariae 2–4 wk after therapy; for patients with no microfilariae in the blood, full doses can be given from day 1; in heavy infections albendazole preferred (slower onset of action and lower risk of encephalopathy), but repeated doses may be required
Mansonella ozzardi	Ivermectin	200 µg/kg PO once	As above; diethylcarbamazine ineffective
Mansonella perstans	Doxycycline	100 or 200 mg/day PO × 6–8 wks	Diethylcarbamazine, mebendazole, ivermectin ineffective against *M. perstans*
Mansonella streptocerca	Diethylcarbamazine (available from CDC)	6 mg/kg/day PO in 3 doses × 12 days	See *Onchocerca volvulus*
	or		
	Ivermectin	150 µg/kg PO once	
Metagonimus yokogawai (intestinal fluke)	Praziquantel	75 mg/kg/day in 3 doses × 1 day	Fecal examinations for eggs 2–4 wk after therapy
Metorchis conjunctus (North American liver fluke)	Praziquantel	75 mg/kg/day PO in 3 doses × 1 day	Fecal examinations for eggs 2–4 wk after therapy
Microsporidia	In some genera and species, treatment not totally effective; see also nitazoxanide under *Giardia lamblia*		
Disseminated			
Encephalitozoon hellem	Albendazole	400 mg BID Children: 15 mg/kg/day in 2 doses (max 400 mg/dose)	Monitor clinical response; dose for children the same for all disseminated infections
Encephalitozoon cuniculi	Albendazole	400 mg BID	Monitor clinical response
Encephalitozoon (Septata) intestinalis	Albendazole	400 mg BID	Monitor clinical response
Pleistophora sp.	Albendazole	400 mg BID	Monitor clinical response; may add itraconazole 400 mg PO once/day
Trachipleistophora spp.	Albendazole	400 mg BID	Monitor clinical response; may add itraconazole 400 mg PO once/day
Anncaliia (Brachiola) vesicularum	Albendazole	400 mg BID	Monitor clinical response; may add itraconazole 400 mg PO once/day

Intestinal			
Enterocytozoon bieneusi	Fumagillin	20 mg PO TID × 14 days	Monitor clinical response; fecal exam for spores 2–4 wk after therapy
Encephalitozoon (*Septata*) *intestinalis*	Albendazole[c]	400 mg BID × 21 days Children: 15 mg/kg/day in 2 doses (max 400 mg/dose)	Monitor clinical response; fecal exam for spores 2–4 wk after therapy
Ocular			
Encephalitozoon hellem	Albendazole plus fumagillin	400 mg BID, eye drops	Monitor clinical response
Encephalitozoon cuniculi	Albendazole plus fumagillin	400 mg BID, eye drops	Monitor clinical response
Vittaforma corneae	Albendazole plus fumagillin	400 mg BID, eye drops	Monitor clinical response
Moniliformis moniliformis	Pyrantel pamoate	11 mg/kg once, repeat twice, 2 wk apart	Fecal exam for eggs 1 mo after therapy
Naegleria fowleri (primary amebic meningoencephalitis)	Amphotericin B (conventional formulation)	0.25 mg/kg/day i.v. over 4–6 h; if tolerated, 0.5 mg/kg i.v. the following day, increasing to 1.5 mg/kg i.v. once/day as tolerated (max 1.5 mg/kg/day)	Monitor clinical response and organisms in CSF; direct wet mounts of CSF and cultures
	Rifampin	10 mg/kg i.v. once/day (max 600 mg/day)	
	Fluconazole	12 mg/kg i.v. once/day	
	Azithromycin	500 mg i.v. once/day Children: 20 mg/kg i.v. once/day (max 500 mg/day)	
Nanophyetus salmincola	Praziquantel	60 mg/kg/day PO in 3 doses × 1 day	Fecal exam for eggs 1 mo after therapy
Oesophagostomum bifurcum	Albendazole	400 mg PO once	Monitor clinical response; pyrantel pamoate may also be effective
	or		
	Pyrantel pamoate	11 mg/kg base (max 1 g) PO once/day × 3 days	
Onchocerca volvulus (river blindness)	Ivermectin	150 µg/kg PO once, repeat every 6–12 mo until asymptomatic	Skin snips for microfilariae 3–6 mo after therapy or sooner if symptoms recur
Opisthorchis viverrini (Southeast Asian liver fluke)	Praziquantel	75 mg/kg/day PO in 3 doses × 2 days	Fecal exam for eggs 1 mo after therapy
	or		
	Albendazole	10 mg/kg/day PO × 7 days	
Paragonimus westermani (lung fluke), *Paragonimus* spp.	Drug of choice: Praziquantel	75 mg/kg/day PO in 3 doses × 2 days	Sputum examination for eggs 2–4 wk after therapy; fecal examinations for eggs 1–2 mo after therapy
	Alternative:		
	Triclabendazole	10 mg/kg PO once or twice	
	or		
	Bithionol	30–50 mg/kg on alternate days × 10–15 doses	

(continued)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a] *(continued)*

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
Plasmodium spp. (*P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, *P. vivax*)			
Oral chemoprophylaxis (prevention) Areas of chloroquine susceptibility	Drug of choice: Chloroquine phosphate	500 mg (300 mg base) once/wk, beginning 1–2 wks before travel, continue weekly for duration of stay and 4 wks after leaving malarious zone Children: 5 mg/kg base once/wk, up to adult dose of 300 mg base; same regimen as adults	No drug guarantees protection against malaria. Travelers should seek medical help if fever develops after returning. Remember to include insect repellents, insecticide-impregnated bed nets and proper clothing, depending on the geographic areas visited.
Areas of chloroquine resistance	Drug of choice: Atovaquone/proguanil	1 adult tab/day, beginning 1–2 days before travel, continue for duration of stay and for 1 wk after leaving malarious area Children: same regimen as adults <5 kg: not indicated 5–8 kg: ½ peds tab/day 9–10 kg: ¾ peds tab/day 11–20 kg: 1 peds tab/day 21–30 kg: 2 peds tabs/day 31–40 kg: 3 peds tabs/day >40 kg: 1 adult tab/day	
	or		
	Doxycycline	100 mg PO daily Children: 2 mg/kg/day PO, up to 100 mg/day	Same regimen as Atovaquone/proguanil
	or		
	Mefloquine	250 mg PO once/wk Children: ≤9 kg: 5 mg/kg salt once/wk 9–19 kg: ¼ tab once/wk 19–30 kg: ½ tab once/wk 31–45 kg: ¾ tab once/wk >45 kg: 1 tab once/wk	
	Alternative: Primaquine phosphate	30 mg base PO daily Children: 0.5 mg/kg base PO daily	
Oral treatment (uncomplicated or mild infection) *P. falciparum* in areas of chloroquine resistance, *P. falciparum* ID or unidentified species	Drug of choice: Atovaquone/proguanil	4 adult tabs PO once/day or 2 adult tabs PO BID × 3 days Children: <5 kg: not indicated 5–8 kg: 2 peds tabs PO once/day × 3 days 9–10 kg: 3 peds tabs PO once/day × 3 days 11–20 kg: 1 peds tab/day PO × 3 days 21–30 kg: 2 peds tabs PO once/day × 3 days 31–40 kg: 3 peds tabs/day × 3 days >40 kg: 1 adult tab/day × 3 days	250 mg/100 mg (1 tablet) daily, beginning 1–2 days before travel, continuing during stay and for 1 wk after leaving area

	or	
	Artemether/lumefantrine	6 doses over 3 days (4 tabs/dose at 0, 8, 24, 36, 48, and 60 h) Children: 6 doses over 3 days at same intervals as adults 5–15 kg: 1 tab/dose >15–25 kg: 2 tabs/dose >25–35 kg: 3 tabs/dose >35 kg: 4 tabs/dose
	or	
	Quinine sulfate	650 mg PO q8h × 3 or 7 days Children: 30 mg/kg/day PO in 3 doses × 3 or 7 days
	plus	
	Doxycycline	100 mg PO BID × 7 days Children: 4 mg/kg/day PO in 2 doses × 7 days
	or plus	
	Tetracycline	250 mg PO QID × 7 days Children: 25 mg/kg/day PO in 4 doses × 7 days
	or plus	
	Clindamycin	20 mg/kg/day PO in 3 doses × 7 days
	Alternative: Mefloquine	750 mg PO followed 12 h later by 500 mg Children: 15 mg/kg PO followed 12 h later by 10 mg/kg
P. vivax in areas of chloroquine resistance, *P. vivax* ID	Drug of choice: Quinine sulfate	650 mg PO q8h × 3 or 7 days Children: 30 mg/kg/day PO in 3 doses × 3 or 7 days
	plus	
	Doxycycline	100 mg PO BID × 7 days Children: 4 mg/kg/day PO in 2 doses × 7 days
	Plus	
	Primaquine phosphate	30 mg base/day PO × 14 days Children: 0.5 mg/kg/day PO × 14 days
	Alternatives:	
	Atovaquone/proguanil	4 adult tabs PO once/day or 2 adult tabs BID, × 3 days Children: <5 kg: not indicated 5–8 kg: 2 peds tabs PO once/day × 3 days 11–20 kg: 1 adult tab PO once/day × 3 days 21–30 kg: 2 adult tabs PO once/day × 3 days 31–40 kg: 3 adult tabs PO once/day × 3 days >40 kg: 4 adult tabs PO once/day × 3 days

(continued)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a] *(continued)*

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
	Mefloquine	750 mg PO followed 12 h later by 500 mg Children: 15 mg/kg PO followed 12 h later by 10 mg/kg	
	either plus		
	Primaquine phosphate	30 mg base/day PO × 14 days Children: 0.5 mg/kg/day PO × 14 days	
All *Plasmodium* spp. in areas with no chloroquine resistance	Drug of choice: Chloroquine phosphate	1 g (600 mg base) PO, then 500 mg (300 mg base) 6 h later, then 500 mg (300 mg base) at 24 and 48 h Children: 10 mg base/kg (max 600 mg base) PO, then 5 mg base/kg 6 h later, then 5 mg base/kg at 24 and 48 h	
Parenteral treatment (severe infection) All *Plasmodium* spp. (chloroquine-sensitive and resistant)	Quinidine gluconate	10 mg/kg IV loading dose (max 600 mg) in normal saline over 1–2 h, followed by continuous infusion of 0.02 mg/kg/min until PO therapy can be started	Uncomplicated or mild malaria may be treated with oral drugs. However, severe malaria (impaired consciousness, >5% parasitemia, shock) requires parenteral drugs.
	or		
	Quinine dihydrochloride	20 mg/kg IV loading dose in 5% dextrose over 4 h, followed by 10 mg/kg over 2–4 h every 8 h (max 1,800 mg/day) until PO therapy can be started	
	or		
	Artesunate plus a second oral drug	2.4 mg/kg/dose IV × 3 days at 0, 12, 24, 48, and 72 h	
Oral treatment (self-presumptive treatment)	Atovaquone/proguanil	4 adult tabs daily once/day or 2 adult tabs BID × 3 days Children: <5 kg: not indicated 5–8 kg: 2 peds tabs once/day × 3 days 9–10 kg: 3 peds tabs once/day × 3 days 11–20 kg: 1 adult tab once/day × 3 days 21–30 kg: 2 adult tabs once/day × 3 days 31–40 kg: 3 adult tabs once/day × 3 days >40 kg: 4 adult tabs once/day × 3 days	A traveler can be given a course of atovaquone/proguanil, mefloquine, or quinine plus doxycycline for presumptive self-treatment of febrile illness. The drug used for self-treatment should be different from that used for prophylaxis. This approach should be used in very rare circumstances when the person cannot promptly receive medical care.
	or		
	Artemether/lumefantrine	6 doses over 3 days (4 tabs/dose at 0, 8, 24, 36, 48, and 60 h)	
	or		
	Quinine sulfate	650 mg PO q8h × 3 or 7 days Children: 30 mg/kg/day in 3 doses × 3 or 7 days	
	plus		
	Doxycycline	100 mg PO BID × 7 days Children: 4 mg/kg/day in 2 doses × 7 days	

Sappinia diploidea (amebic meningoencephalitis)	Azithromycin plus	250 mg/day PO × 1 wk	Combined therapy with surgical resection of CNS lesion
	Pentamidine (i.v.) plus	300 mg/day, 6 wk	
	Itraconazole plus	200 mg BID, 25 wk	
	Flucytosine	2.75 g QID, 25 wk	
	Alternatives:		
	Ivermectin	200 µg/kg PO once	
	or		
	10% Crotamiton	Topically once/day × 2	
Sarcoptes scabiei (itch mite)	Drug of choice: 5% Permethrin	Topically; massage from head to soles of feet; wash off after 8–14 h	Monitor clinical course; may need mild steroid cream in sensitive individuals; may require retreatment in 10–14 days; ivermectin plus scabicides recommended for crusted scabies in immunocompromised patients
Schistosoma haematobium	Praziquantel	40 mg/kg/day in 2 doses × 1 day	Urine examinations for eggs 1 mo after therapy; bladder or rectal biopsy; treatment failure reported
Schistosoma intercalatum	Praziquantel	40 mg/kg/day in 2 doses × 1 day	Fecal examinations for eggs 1 mo after therapy; rectal biopsy
Schistosoma japonicum	Praziquantel	60 mg/kg/day in 3 doses × 1 day	Fecal examinations for eggs 1 mo after therapy; rectal biopsy
Schistosoma mansoni	Drug of choice: Praziquantel	40 mg/kg/day in 2 doses × 1 day	Fecal examinations for eggs 1 mo after therapy; rectal biopsy
	Alternative: Oxamniquine	15 mg/kg once Children: 20 mg/kg/day in 2 doses × 1 day	Contraindicated in pregnancy; East Africa, increase dose to 30 mg/kg; South Africa, increase dose to 30 mg/kg × 2 days
Schistosoma mekongi	Praziquantel	60 mg/kg/day in 3 doses × 1 day	Fecal examinations for eggs 1 mo after therapy; rectal biopsy
Strongyloides stercoralis	Drug of choice: Ivermectin	200 µg/kg/day PO × 2 days	Fecal examinations and/or agar plate culture 1 mo after therapy
	Alternative: Albendazole	400 mg BID × 7 days	
Taenia saginata (beef tapeworm)	Drug of choice: Praziquantel	5–10 mg/kg PO once	Fecal examinations for eggs or proglottids 1 mo after therapy
Taenia solium (pork tapeworm) Adult tapeworms	Alternative: Niclosamide	2 g PO once Children: 50 mg/kg PO once	Availability problems
Toxocara canis, T. cati (visceral larva migrans)	Albendazole	400 mg BID PO × 5 days	Monitor clinical course; some consultants recommend treating for 20 days; severe symptom or eye involvement, treat for 2–4 wks and can add corticosteroids
	or		
	Mebendazole	100–200 mg PO BID × 5 days	

(continued)

TABLE 36.1 Drugs and dosages for treating parasitic infections[a] *(continued)*

Organism or infection	Drug	Dosage[b]	Follow-up[b] and comments
Toxoplasma gondii	Drugs of choice:		
	Pyrimethamine	200 mg PO × 1 day, then 50–75 mg/day PO × 3–6 wks	Monitor clinical course and serology; use only after first trimester
		Children: 2 mg/kg/day PO × 2 days, then 1 mg/kg/day (max 25 mg/day) × 3–6 wks	
	plus		
	Sulfadiazine	1–1.5 g PO QID × 3–6 wks	
		Children: 100–200 mg/kg divided every 6 h PO × 3–6 wks	
	or plus		
	Clindamycin	1.8–2.4 g/day i.v. or PO in 3 or 4 doses	
		Children: 5–7.5 mg/kg/day i.v. or PO in 3 or 4 doses (max 600 mg/dose)	
	or plus		
	Atovaquone	1,500 mg PO BID	
	Alternatives:		
	TMP/SMX	TMP 15–20 mg/kg/day SMX 75–100 mg/kg/day PO or i.v. in 3 or 4 doses	
	or		
	Spiramycin (alternative)	3–4 g/day × 3–4 weeks	Can be used during first-trimester infection
Trichinella spiralis, other Trichinella spp.	Drugs of choice:		
	Mebendazole	200–400 mg TID × 3 days, then 400–500 mg TID × 10 days	Monitor clinical course; perform muscle biopsy in severe cases; steroids for severe symptoms
	plus		
	Corticosteroids	Prednisone: 20–60 mg/day PO daily × 10–15 days	Use for severe symptoms
	Albendazole (alternative)	400 mg PO BID × 8–14 days	
Trichomonas vaginalis	Tinidazole	2 g PO once Children: 50 mg/kg once (max 2 g)	Pelvic examination 1 mo after therapy; sexual partners should be treated simultaneously
	or		
	Metronidazole	2 g PO once Children: 15 mg/kg/day PO in 3 doses × 7 days	Take with food to minimize adverse side effects; can be used with metronidazole-resistant strains
Trichostrongylus spp.	Drug of choice: Pyrantel pamoate	11 mg/kg base PO once (max 1 g)	Fecal examinations 2–4 wk after therapy
	Alternatives:		
	Mebendazole	100 mg PO BID × 3 days	
	or		
	Albendazole	400 mg PO once	
Trichuris trichiura (whipworm)	Drug of choice: Albendazole	400 mg PO × 3 days	Fecal examinations 2–4 wk after therapy
	Alternatives:		
	Mebendazole	100 mg PO BID × 3 days	
	or		
	Ivermectin	200 µg/kg/day PO × 3 days	

Tropical pulmonary eosinophilia	Diethylcarbamazine (available from CDC)	6 mg/kg/day in 3 doses × 12–21 days	Monitor clinical course; if relapse occurs, re-treat
Trypanosoma brucei gambiense (West African sleeping sickness), early stages (hemolymphatic)	Drug of choice: Pentamidine Alternative: Suramin	4 mg/kg/day i.m. or i.v. × 7 days 100 mg (test dose) i.v., then 1 g i.v. on days 1, 3, 5, 14, and 21 Children: 2 mg/kg (test dose) i.v., then 20 mg/kg i.v. on days 1, 3, 5, 14, and 21	Thick and thin blood smears 1–2 mo after therapy
Trypanosoma brucei rhodesiense (East African sleeping sickness), early stages (hemolymphatic)	Suramin	100 mg (test dose) i.v., then 1 g i.v. on days 1, 3, 5, 14, and 21 Children: 2 mg/kg (test dose) i.v., then 20 mg/kg i.v. on days 1, 3, 5, 14, and 21	
T. brucei gambiense and *T. brucei rhodesiense,* late stage with CNS disease	Melarsoprol (first choice for Rhodesian form)	2.2 mg/kg/day i.v. × 10 days Children: 18–25 mg/kg total over 1 mo; initial dose of 0.36 mg/kg i.v., increasing gradually to max of 3.6 mg/kg at intervals of 1–5 days for total of 9–10 doses	CSF analysis during and after therapy
	or		
	Eflornithine (first choice for Gambian form)	400 mg/kg/day i.v. in 4 doses × 14 days	Highly effective for *T. brucei gambiense* but variable for *T. brucei rhodesiense*
	or		
	Eflornithine plus nifurtimox	15 mg/kg/day PO in 3 doses × 10 days	
Trypanosoma cruzi (American trypanosomiasis, Chagas' disease)	Benznidazole	5–7 mg/kg/day PO in 2 divided doses × 60 days Children ≤12 yr: 10 mg/kg/day PO in 2 doses × 60 days >12 yrs: 5–7 mg/kg/day PO in 2 doses × 60 days	Thick and thin blood smears or xenodiagnosis 1–2 mo after therapy; monitor serology and EKG
	or		
	Nifurtimox	8–10 mg/kg/day PO in 3–4 doses × 90 days Children: 1–10 yr: 15–20 mg/kg/day PO in 4 doses × 90 days 11–16 yr: 12.5–15 mg/kg/day PO in 4 doses × 90 days	
Wuchereria bancrofti	Diethylcarbamazine (available from CDC)	6 mg/kg/day PO in 3 doses × 1 or 12 days	Thick blood smears, Knott concentration, and membrane filtration of blood after 2–4 wks

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[a] It is **very important** to thoroughly review drug information prior to use and to review published commentary concerning availability, contraindications, and monitoring requirements. Specific information can also be obtained online by checking *The Medical Letter*, vol 11, 2013 (The Medical Letter, New Rochelle, NY, http://www.medletter.com/freedocs/parasitic.pdf [accessed June 23, 2014]).

[b] BID, twice a day; TID, three times a day; QID, four times a day. CDC, Centers for Disease Control and Prevention; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; DS tab, double-strength tablet; EIA, enzyme immunoassay; EKG, electrocardiogram; i.m., intramuscular; i.v., intravenous; max, maximum; peds, pediatric; PHMB, polyhexamethylene biguanide; PO, orally; PC, with meals; SMX, sulfamethoxazole; TMP, trimethoprim.

[c] See also nitazoxanide.

TABLE 36.2 Antiparasitic drugs[a]

Drug	Manufacturer	Trade name and comments
Albendazole	Amedra	Albenza
Aminosidine		See Paromomycin
Amphotericin B	X-Gen	Fungizone, generics
Amphotericin B, liposomal	Gilead	AmBisome
Artemether[b]	Arenco, Belgium	Artenam; not available in the United States
Artemether/lumefantrine	Novartis	Coartem, Riamet
Artesunate[e]	Guilin No. 1 Factory, People's Republic of China	Not available in the United States
Atovaquone	GlaxoSmithKline	Mepron
Atovaquone-proguanil	GlaxoSmithKline	Malarone
Azithromycin	Pfizer	Zithromax
Bacitracin	Many manufacturers	
Benznidazole[c]	Roche, Brazil	Rochagan; not available in the United States
Bithionol[b]		Bitin; available through CDC Drug Service
Chloroquine HCl, chloroquine phosphate	Sanofi, others	Aralen, generics
Clarithromycin	Abbvie	Biaxin, generics
Clindamycin	Pfizer, others	Cleocin, generics
Crotamiton	Ranbaxy	Eurax
Dapsone	Jacobus	Generics
Diethylcarbamazine citrate (DEC)[c]		Hetrazan; available through CDC Drug Service
Dihydroartemisin/piperaquine	Sanofi	Euratesim
Diloxanide furoate[b]	Boots, United Kingdom	Furamide, Entamide; not available in the United States
Doxycycline	Pfizer, others	Vibramycin; also available generically
Eflornithine (difluoromethylornithine, DFMO)[c]	Sanofi	Ornidyl; available through CDC Drug Service
Fluconazole	Pfizer	Diflucan, generics
Flucytosine	Valeant	Ancobon
Fumagillin[b]	Leiter's Park Avenue Pharmacy, San Jose, CA (800-292-6773) (www.leiterrx.com); Sanofi, France	Flisint, not available in the United States
Furazolidone[b]		Furoxone; not available in the United States
Iodoquinol	Glenwood, others	Yodoxin; generics
Itraconazole	Janssen	Sporanox
Ivermectin	Merck, Sanofi	Stromectol, Sklice
Ketoconazole	Janssen, others	Nizoral; generics
Lumefantrine/Artemether	Novartis	Coartem, Riamet
Levamisole	Janssen	Ergamisol
Malathion	Taro	Ovide
Mebendazole		Generics
Mefloquine		Generics
Meglumine antimoniate[b]	Sanofi, France	Glucantime; not available in the United States
Melarsoprol[c]		Mel-B; available through CDC Drug Service
Metronidazole	Pfizer/Searle	Flagyl; generics
Metronidazole IV	Baxter	
Miconazole[b]		Monistat
Miltefosine[c,d]	Paladin, Canada	Impavido, Miltex; not available in the United States
Niclosamide[b]		Yomesan; not available in the United States
Nifurtimox[c]	Bayer HealthCare	Lampit; available through CDC Drug Service

(continued)

TABLE 36.2 *(continued)*

Drug	Manufacturer	Trade name and comments
Nitazoxanide	Romark	Alinia
Ornidazole[b]		Tiberal; not available in the United States
Oxamniquine[b]	Pfizer	Vansil; not available in the United States
Paromomycin	Sun Pharma	Oral: generics Topical: Leshcutan[b]
Pentamidine isethionate	APP Pharmaceuticals	Pentam 300, NebuPent
Permethrin	Insight, Bayer	Nix
	Prestium Pharma	Elimite, generics
Piperaquine/dihydroartemisin	Sigma Tau, Italy	Euratesim
Praziquantel	Bayer	Biltricide
Primaquine phosphate USP	Sanofi-Aventis	
Proguanil[b]		Paludrine; not available in the United States
Proguanil-atovaquone	GlaxoSmithKline	Malarone
Propamidine isethionate[b]	Aventis	Brolene; not available in the United States
Pyrantel pamoate	Quartz Specialty Pharmaceuticals, Reese	Pin-X, Reese's Pinworm Medicine
Pyrethrins and piperonyl butoxide	Bayer	Rid; generics
Pyrimethamine USP[b]	Amedra	Daraprim
Quinidine gluconate	Generics	
Quinine dihydrochloride		
Quinine sulfate	AR Scientific	Qualaquin
Rifampin	Sanofi	Rifadin; generics
Sodium stibogluconate[c]	GlaxoSmithKline, United Kingdom	Pentostam; available through CDC Drug Service
Spiramycin[e]	Sanofi	Rovamycine
Sulfadiazine	Generics	
Suramin sodium[c]	Bayer, Germany	Germanin; available through CDC Drug Service
Thiabendazole	Merck	Mintezol
Tinidazole	Mission Pharmaceuticals	Tindamax
TMP/Sulfa (TMP/SMX)	Sun Pharma	Bactrim; generics
Triclabendazole[b]	Novartis	Egaten; not available in the United States
Trimetrexate[b]		Neutrexin

[a] Information from reference 1.

[b] Not available in the United States; may be available through a compounding pharmacy such as Expert Compounding Pharmacy, 6744 Balboa Blvd., Lake Balboa, CA 91406 (800-247-9767) or Medical Center Pharmacy, New Haven, CT (203-688-7064). Other compounding pharmacies may be found through the National Association of Compounding Pharmacies (800-687-7850) or the Professional Compounding Centers of America (800-331-2498, www.pccarx.com).

[c] Available from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, GA 30333; 404-639-3670 (evenings, weekends, or holidays: 770-488-7100; fax: 404-639-3717).

[d] Available from the CDC for treatment of infections with free-living amebae; available from the manufacturer through an Investigational New Drug (IND) application from the U.S. Food and Drug Administration (FDA) for treatment of other infections.

[e] Available through an IND request from the FDA. IND request paperwork is available through the CDC.

Amphotericin B (Fungizone) (X-Gen)

Preparation

Fungizone is supplied in vials containing 50 mg of amphotericin B, 4 mg of sodium deoxycholate, and 20.2 mg of sodium phosphate as a buffer. It should be stored in the refrigerator and protected from light. It may be reconstituted by the addition of 10 ml of sterile water for injection USP, without a bacteriostatic agent, to make a 5-mg/ml solution. This may be further diluted by adding 1 ml of the 5-mg/ml solution to 50 ml of 5% glucose solution to make a final concentration of 0.1 mg of amphotericin per ml. The pH of the solution should be above 4.2. Amphotericin B solutions have been diluted with cerebrospinal fluid before intrathecal administration.

Administration

Therapy is usually initiated with a test dose of 0.25 mg/kg/day and gradually increased to a maximum dose of 1.5 mg/kg/day. It must be given slowly i.v., usually over a 6-

h period. Alternate-day therapy may be better tolerated. For patients with severe life-threatening infections, therapy may have to be initiated with high doses. It may be given intrathecally in the lumbar or cisternal areas. Low doses should be given if the drug is administered intraventricularly.

Toxicity

Nausea, vomiting, fever, and phlebitis are common. Some degree of renal impairment usually occurs and is dose related. Renal function (blood urea nitrogen or serum creatine) should initially be monitored daily. Other side effects include anaphylaxis, hypokalemia, hypotension, anemia, and cardiac dysrhythmias. The side effects are much milder when amphotericin B is delivered in liposomes (AmBisome). This preparation is more expensive but makes the drug more tolerable, especially in patients with known renal failure. The main side effects of liposomal preparations are hypersensitivity reactions.

Contraindications

It may be necessary to pretreat the patient with antiemetic agents or antipyretic drugs. For patients with known hypersensitivity to the drug, it should be used only in life-threatening situations. Concomitant use of other nephrotoxic drugs should be avoided. Extreme caution should be used when the patient is taking other nephrotoxic drugs such as aminoglycosides, high-dose i.v. acyclovir, ganciclovir, foscarnet, and pentamidine. Corticosteroids and adrenocorticotropic hormone may lead to hypokalemia. The drug may increase the bone marrow toxicity of ganciclovir. Probenecid may increase the levels of amphotericin B.

Artemether (Artenam) (Aarenco, Belgium)

Preparation

Artemether is oil soluble and available in tablet, capsule, or intramuscular-injection forms. Artesunate is water soluble and can be given i.v., intramuscularly, orally, or by suppository. The activity of these artemisinin derivatives decreases after 1 to 2 h. To counter this drawback, artemisinin is given alongside lumefantrine to treat uncomplicated falciparum malaria. Lumefantrine has a half-life of about 3 to 6 days. Such a treatment is called ACT (artemisinin-based combination therapy); other examples are artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, and artesunate-sulfadoxine/pyrimethamine. The artemisinin-based combinations artemether-lumefantrine and artesunate-mefloquine remain highly effective and elicit equivalent therapeutic responses in the treatment of highly drug-resistant falciparum malaria.

Administration

The drugs are given as 250-mg tablets of artemisinin and artesunate orally; they are also available as capsules containing 100 mg of artemisinin. Drugs that are available include artemether-lumefantrine (Coartem) (Novartis).

Toxicity

The artemisinin derivatives are well tolerated, with no serious toxicity or adverse effects. With artemether/lumefantrine, there may be abdominal pain, anorexia, headache, dizziness, diarrhea, vomiting, nausea, palpitations, arthralgia, myalgia, asthenia, fatigue, pruritus, rash, sleep disorder, and cough. With artemether, there may be occasional neurological toxicity.

Contraindications

These drugs should be used with caution in individuals with hepatic or renal impairment. They should be avoided in pregnancy due to insufficient safety information.

Comment

Artemisia annua is a plant with a strong aroma, containing camphor and essential oils. It is a robust plant that grows in many areas of the world. However, only plants grown under special agricultural and geographic conditions contain artemisinin. The best high-yielding samples have been collected from the steep hills at altitudes over 4,500 feet around Youyang County, City of Chongqing, in Szechuan Province, China.

These drugs act mainly against the asexual erythrocytic stages of malaria. High concentrations of iron are found in red blood cells, which is also where the malaria parasites are found. When the compound enters the red blood cell, it releases free radicals, which are highly destructive to the parasites. An alternative theory suggests that artemisinins inhibit a calcium transporter in the parasite and, by specifically inhibiting this target, kill the parasites. The peroxide group in artemisinins is still crucial to activity, but the target is highly selected rather than a result of “explosion” into activity by free radicals. These drugs are also active against gametocytes by about 90% but are not effective against the intrahepatic stage of *P. vivax* and *P. ovale*.

Atovaquone (Mepron) (GlaxoSmithKline)

Preparation

Atovaquone suspension is a formulation of microfine particles of the drug. The atovaquone particles, reduced in size to facilitate absorption, are significantly smaller than those in the previously marketed tablet formulation. The suspension is for oral administration and is bright yellow with a citrus flavor, containing 150 mg of atovaquone per ml or 750 mg of atovaquone in each teaspoonful (5 ml).

Administration

The drug is given orally with meals; it is used to treat *Babesia* spp. and *Pneumocystis jirovecii* (now classified with the fungi) infections. Administering atovaquone with food enhances its absorption by approximately 2-fold.

Toxicity

Mepron may cause nausea, vomiting, diarrhea, and skin rashes, as well as sweating, flu syndrome, pain, sinusitis, insomnia, depression, and myalgia. Monitor for hepatitis, elevated liver function tests and liver failure.

Contraindications

Mepron should not be given to anyone who has developed or has a history of life-threatening reactions to any of the components of the formulation. If it is necessary to treat patients with severe hepatic or renal impairment, caution is advised and administration should be closely monitored. This drug should be used in pregnancy only if the potential benefits outweigh the potential risks to the fetus. It should not be used during lactation.

Atovaquone-Proguanil (Malarone) (GlaxoSmithKline)

Preparation

Malarone (atovaquone and proguanil hydrochloride) is a fixed-dose combination of the antimalarial agents atovaquone and proguanil hydrochloride. Each tablet contains 250 mg of atovaquone and 100 mg of proguanil hydrochloride, and each pediatric tablet contains 62.5 mg of atovaquone and 25 mg of proguanil hydrochloride.

Administration

The drug is given orally with meals or a milky drink; atovaquone and cycloguanil (an active metabolite of proguanil) are active against the erythrocytic and exoerythrocytic stages of *Plasmodium* spp. Malarone is indicated for the prophylaxis of *P. falciparum* malaria, including in areas where chloroquine resistance has been reported. It is also indicated for the treatment of acute, uncomplicated *P. falciparum* malaria and is effective in regions where the drugs chloroquine, halofantrine, mefloquine, and amodiaquine may have unacceptable failure rates, presumably due to drug resistance.

Toxicity

Malarone may cause abdominal pain, nausea, vomiting, headache, diarrhea, asthenia, anorexia, and dizziness. About 5 to 10% of patients have transient asymptomatic elevations in transaminase and amylase levels.

Contraindications

Malarone should not be given to anyone who has developed or has a history of life-threatening reactions to any of the components of the formulation. Rare cases of anaphylaxis following treatment with atovaquone-proguanil have been reported. Due to the lack of safety data, the drug should be avoided during pregnancy or lactation or in patients with renal or hepatic impairment. Malarone has not been evaluated for the treatment of cerebral malaria or other severe manifestations of complicated malaria, including hyperparasitemia, pulmonary edema, or renal failure.

Comment

The drug interferes with two different pathways involved in the biosynthesis of pyrimidines required for nucleic acid replication. Atovaquone is a selective inhibitor of parasite mitochondrial electron transport, while the metabolite of proguanil hydrochloride, cycloguanil, is a dihydrofolate reductase inhibitor. Malarone is effective against asexual and sexual forms of *P. falciparum* and is used for prophylaxis and treatment. Unfortunately, resistance and drug failures are being reported for *P. falciparum*. Although the drug is also effective for treating *P. vivax* and *P. ovale,* primaquine is required to prevent relapses from the liver.

Benznidazole (Rochagan) (Roche, Brazil)

Preparation

Benznidazole is a 2-nitroimidazole derivative and is provided as 100-mg tablets. It is used to treat acute Chagas' disease but is not available in the United States.

Administration

The drug should be taken with meals, preferably after breakfast and after supper, to decrease the chance of stomach upset.

Toxicity

Side effects are seen in many individuals and include nausea, vomiting, abdominal pain, peripheral neuropathy, severe skin reactions, bone marrow suppression, and psychic disturbances.

Contraindications

Alcohol should be avoided during treatment. The drug has been noted to cause lymphoma in animal models and is mutagenic. Its use is not recommended during the first trimester of pregnancy. Benznidazole should not be used with anticoagulants or in patients who have renal or hepatic impairment. Although this side effect is rare, benznidazole can lower the number of white blood cells, increasing the patient's chance of getting an infection. It can also lower the number of platelets, which are necessary for proper blood clotting.

Comment

Benznidazole inhibits protein and RNA synthesis in *Trypanosoma cruzi;* it causes increased phagocytosis, cytokine release, and production of reactive mitogen intermediates that lead to death of the parasites.

Bithionol (Bitin) (CDC)

Preparation

Bitin is supplied as 500-mg capsules, which must be obtained from CDC. The drug is used to treat several trematode infections.

Administration

The capsules are taken orally with meals to minimize stomach irritation.

Toxicity

Patients may experience anorexia, nausea, vomiting, abdominal discomfort, diarrhea, dizziness, and headaches.

Contraindications

Bitin may cause leukopenia and toxic hepatitis, and photosensitization has occurred.

Comment

Bithionol, an investigational drug, is a phenolic substance structurally related to hexachlorophene. It is used as an alternative drug to praziquantel for treating *Paragonimus* infections. It can be used to treat extrapulmonary infections with *Paragonimus*, but multiple courses may be required for cure. Surgery may be needed to excise skin lesions or, rarely, brain cysts. Bithionol is also used to treat *Fasciola hepatica* infections. Its use is limited to treating either of these infections in patients who are unable to tolerate praziquantel due to previous reactions or in whom a previous course of praziquantel was ineffective.

Chloroquine Phosphate (Aralen) (Sanofi, Others)

Preparation

Aralen is supplied in 250- and 500-mg tablets of the diphosphate salt (500 mg of salt = 300 mg of base) and as a hydrochloride for parenteral injection in 5-ml vials containing 40 mg of base per ml. A liquid preparation (Nivaquine) is available outside the United States and is used as an antimalarial agent.

Administration

Aralen is given orally as per protocol for chemoprophylaxis or treatment of an acute attack. Pills can be ground up and placed in sweetened juice or fruit for administration to young children.

Toxicity

Side effects may be dose related and include gastrointestinal symptoms, skin rash, insomnia, headache, and nervousness. Rare reactions such as toxic psychosis and retinal damage, blood dyscrasias, and photophobia may occur.

Contraindications

Relative contraindications include liver, hematologic, and/or neurologic disease; psoriasis; and porphyria. It may be used in pregnant women when necessary for protection. Use of this drug is contraindicated in the presence of retinal or visual field changes either attributable to 4-aminoquinoline compounds or to any other etiology and in patients with known hypersensitivity to 4-aminoquinoline compounds. Parenteral chloroquine may cause hypotension, shock, and sudden death when given to young children.

Comment

Chloroquine is active against the erythrocytic forms of *Plasmodium vivax*, *P. malariae*, and susceptible strains of *P. falciparum* (but not the gametocytes of *P. falciparum*). It is not effective against exoerythrocytic forms of the parasite. Resistance of *P. falciparum* to chloroquine is widespread, and cases of *P. vivax* resistance have been reported.

Crotamiton (Eurax) (Ranbaxy)

Preparation

Eurax is supplied as a 10% cream or lotion and is used to treat scabies (*Sarcoptes scabiei*).

Administration

The cream or lotion is applied to the whole body surface below the chin after bathing and drying. A second application is advisable after 24 h. Reinfection from contaminated clothing and bed linens should be avoided.

Toxicity

The drug can cause irritation and sensitivity reactions, which may include rash or conjunctivitis.

Contraindications

The drug should not be used in the presence of acute exudative dermatitis and should not be applied near the eyes.

Dapsone (Jacobus)

Preparation

Dapsone is supplied as 25- and 100-mg scored tablets and used to treat *Plasmodium falciparum* (combination therapy) and *Pneumocystis jirovecii* (now classified with the fungi) infections.

Administration

Dapsone should be taken orally, and the dosage should be individually titrated.

Toxicity

Dapsone has been associated with frequent rashes, transient headaches, gastrointestinal irritation, anorexia, and infectious mononucleosis-like syndrome. The drug is carcinogenic in animal models. Complete blood counts should be performed frequently because the drug has been associated with death from agranulocytosis, aplastic anemia, and other blood dyscrasias.

Contraindications

The drug should not be given to patients with hypersensitivity to dapsone. Hemolysis and Heinz body formation may be exaggerated in individuals with a glucose-6-phosphate dehydrogenase (G6PDH) deficiency, or methemoglobin reductase deficiency, or hemoglobin M. This reaction is frequently dose related. Dapsone should be given with caution to these patients or to patients who have been exposed to other agents or conditions such as infection or diabetic ketosis capable of producing hemolysis.

Diethylcarbamazine Citrate USP (Hetrazan) (CDC)

Preparation

Diethylcarbamazine is a piperazine derivative and is supplied in 50-mg tablets. It is used to treat filariasis and is effective against microfilariae; it is available from CDC. Hetrazan is effective against *Wuchereria bancrofti*, *Brugia malayi*, *B. timori*, *Onchocerca volvulus*, *Loa loa*, and *Mansonella streptocerca* but has little effect on *M. perstans* or *M. ozzardi*.

Administration

Tablets are scored to provide the appropriate dose. Medication should be taken after meals, adhering to a strict dosage schedule.

Toxicity

Reactions occur frequently and include headache, weakness, malaise, fever, joint pain, anorexia, nausea, and vomiting. These generally subside as therapy continues. Mild to severe reactions, including fever, rash, lymphadenopathy, conjunctivitis, leukocytosis, and eosinophilia, may occur in response to the parasite's death. Severe reaction may require steroid therapy and antihistamines.

Contraindications

Treatment of patients with heavy microfilaremia due to loiasis may cause allergic encephalitis, nephritis, or myocarditis. Patients with many microfilariae or onchocerciasis in ocular tissues may experience keratitis, chorioretinitis, and optic neuritis. Dose reductions are generally required in patients with renal impairment. The drug should probably be avoided in pregnancy but is not excreted in breast milk.

Comment

Diethylcarbamazine citrate, an investigational drug, is an anthelmintic agent that does not resemble other antiparasitic compounds. It is a synthetic organic compound which is highly specific for several parasites and does not contain any toxic metallic elements. It is used to treat patients with certain filarial infections, including lymphatic filariasis (*W. bancrofti*, *B. malayi*, or *B. timori*), tropical pulmonary eosinophilia, and loiasis (*Loa loa*). Treatment of lymphatic filariasis is problematic. Diethylcarbamazine kills microfilariae but only a variable proportion of adult worms. Another drug, ivermectin, rapidly reduces microfilaremia levels and may inhibit larval development in mosquitoes, but it does not kill adult worms and is not very effective against brugian filariasis.

Diloxanide Furoate (Furamide, Entamide) (Boots, England)

Preparation

Diloxanide furoate is supplied as scored 500-mg tablets and is used to treat amebiasis; it is not available in the United States. The drug is hydrolyzed in the bowel to the active compound, diloxanide, which is >90% absorbed.

Administration

Furamide is given by mouth with repeated treatments as necessary.

Toxicity

The most common adverse effect is flatulence. Other side effects are seen, including vomiting, pruritus, and urticaria.

Contraindications

The use of Furamide is generally not recommended during pregnancy or lactation.

Comment

The drug helps clear the bowel of *Entamoeba histolytica* cysts; however, it is not effective for treating invasive disease in the bowel wall or liver. For this reason, it is often given with a 5-nitroimidazole.

Eflornithine (Difluoromethylornithine, Ornidyl) (Sanofi) (CDC)

Preparation

The drug is available as a 200-mg/ml solution, and is used to treat African trypanosomiasis.

Administration

Ornidyl is given as a continuous i.v. infusion or orally; however, oral administration is usually followed by diarrhea. Eflornithine crosses the blood-brain barrier; thus it is beneficial for central nervous system disease associated with African trypanosomiasis.

Toxicity

Side effects occur in about 40% of patients; they include anemia, leukopenia, thrombocytopenia, diarrhea, abdominal discomfort, hearing loss, vomiting, dizziness, arthralgia, and numbness of the limbs.

Contraindications

Ornidyl should not be given with other i.v. drugs. It may worsen hemolytic disorders. Due to insufficient safety data, the drug should not be used during pregnancy or lactation.

Comment

Eflornithine inhibits ornithine decarboxylase, an enzyme required for the formation of polyamines, thus leading to inhibition of parasite growth. However, it is ineffective as monotherapy for *T. brucei rhodesiense.*

Fumagillin (Fumidil-B)

Preparation

The drug is supplied as a 10-mg/ml suspension in physiologic saline. Ocular lesions due to *Encephalitozoon hellem* and *Encephalitozoon cuniculi* have responded to fumagillin eye drops prepared from Fumidil-B used to control microsporidial infection in honeybees. For lesions due to *Vittaforma corneae*, topical therapy is generally not effective and keratoplasty may be required. It has also been used to treat infections with *Enterocytozoon bieneusi* in the gastrointestinal tract. The drug is available in limited supply from CDC and the World Health Organization.

Administration

Fumagillin should be applied topically once an hour until clinical improvement is demonstrated.

Toxicity

The soluble salt is toxic and should not be ingested; topical application is not toxic to the cornea.

Contraindications

No information is available.

Comment

Relapse may occur on discontinuation of fumagillin therapy.

Furazolidone (Furoxone)

Preparation

Furoxone is supplied as 100-mg tablets or as a flavored suspension of 50 mg/liter and is used to treat giardiasis. This drug is not available in the United States.

Administration

The drug is given orally. Apparently furazolidone is more effective by 50% than metronidazole in inhibiting *in vitro* cyst differentiation of *Giardia lamblia.*

Toxicity

Furoxone can cause polyneuritis, nausea, vomiting, dizziness, drowsiness, and general malaise. Skin rashes and agranulocytosis have occasionally been reported. Furazolidone is chemically similar to nitrofurantoin, which is well known to cause pulmonary hypersensitivity reactions; furazolidone may also induce pulmonary hypersensitivity reactions, and clinicians should be aware of this potentially serious adverse effect.

Contraindications

The drug should not be given to individuals hypersensitive to furazolidone or to infants younger than 1 month. Hemolytic anemia can occur in patients with G6PDH deficiency. Alcohol should be avoided during treatment, because disulfiram-like reactions have been reported. Furazolidone is a monoamine oxidase inhibitor and should not be given concomitantly with other similar drugs.

Iodoquinol/Diiodohydroxyquin (Yodoxin) (Glenwood, Others)

Preparation

Iodoquinol/diiodohydroxyquin is supplied as 210- and 650-mg tablets and is used as an amebicide against *Entamoeba histolytica.* The drug is considered effective against the trophozoite and cyst forms.

Administration

The drug should be given with meals. For young children, a suspension may be made by mixing the pulverized tablet with cherry syrup to give a suspension containing 200 to 400 mg/5 ml. The drug is poorly absorbed.

Toxicity

There may be various forms of skin eruptions, urticaria, and pruritus. There may also be nausea, vomiting, abdominal cramps, diarrhea, and pruritus ani. Fever, chills, headache, vertigo, and enlargement of the thyroid have been reported. Optic neuritis, optic atrophy, and peripheral neuropathy have been reported in association with prolonged high-dosage 8-hydroxyquinoline therapy.

Contraindications

Iodoquinol should not be given to patients with known hypersensitivity to iodine or any 8-hydroxyquinoline compound, with renal or thyroid disease, or with severe liver disease not due to amebiasis. Iodoquinol should be used with caution in patients with thyroid disease. Protein-bound serum iodine levels may be increased during treatment with iodoquinol and may interfere with certain thyroid function tests. These effects may persist for as long as 6 months after discontinuation of therapy; this effect is unrelated to thyroid function. The drug should be withdrawn if hypersensitivity reactions occur. Severe neurologic damage, including optic neuritis, optic atrophy, and peripheral neuropathy, has occurred with prolonged high doses of this drug. The recommended doses and duration of therapy should not be exceeded. There may be persistent elevation of the protein-bound iodine level months after therapy. Because of the limited safety data available, this drug should probably be avoided during pregnancy or lactation.

Comment

The drug is thought to act by inactivating parasitic enzymes and inhibiting organism multiplication. Although it is effective for treating intestinal organisms, it is not effective for treating invasive disease in the bowel wall or liver; it is often combined with a 5-nitroimidazole. It has documented activity against *Balantidium coli*, *Dientamoeba fragilis*, *Giardia lamblia*, and *Blastocystis* spp.

Ivermectin (Stromectol, Sklice) (Merck, Sanofi)

Preparation

The drug is available in 3-mg or 6-mg tablets and is a semisynthetic macrolide used to treat nondisseminated strongyloidiasis and filariasis, particularly onchocerciasis (no activity against adult *Onchocerca volvulus* parasites). It is also now used for the treatment of ectoparasitic infestations such as scabies and lice, although it has no effect on the eggs (nits).

Administration

The drug is given orally with water 1 h before breakfast and is rapidly absorbed after ingestion.

Toxicity

The drug can cause fever, headache, rash, increased pruritus, joint and muscle pain, and tachycardia. A Mazzotti-type reaction has been seen in patients with onchocerciasis. Most adverse effects are a result of the host immune response to destruction of the parasites rather than effects of the drug itself. When used for ectoparasites, monitor for conjunctivitis, ocular hyperemia, eye irritation, dandruff, dry skin, and skin burning sensation.

Contraindications

Ivermectin should not be given to pregnant or lactating women, because it has been noted to cause birth defects in mice. Historical data have shown that microfilaricidal drugs, such as diethylcarbamazine citrate, might cause cutaneous and/or systemic reactions of varying severity (the Mazzotti reaction) and ophthalmologic reactions in patients with onchocerciasis. These reactions are probably due to allergic and inflammatory responses to the death of microfilariae. Patients treated with Stromectol for onchocerciasis may experience these reactions in addition to clinical adverse reactions possibly, probably, or definitely related to the drug itself.

Comment

Ivermectin is active against various life cycle stages of many but not all nematodes. It is active against the tissue microfilariae of *Onchocerca volvulus* but not against the adult form. The adult parasites reside in subcutaneous nodules which are infrequently palpable. Surgical excision of these nodules (nodulectomy) may be considered in the management of patients with onchocerciasis, since this procedure eliminates the microfilaria-producing adult parasites.

Ivermectin may be highly effective against *Strongyloides* and produces fewer side effects than thiabendazole. Compounds of the class bind selectively and with high affinity to glutamate-gated chloride ion channels which occur in invertebrate nerve and muscle cells. This leads to an increase in the permeability of the cell membrane to chloride ions with hyperpolarization of the nerve or muscle cell, resulting in paralysis and death of the parasite.

The drug is also effective against the microfilariae of *Wuchereria bancrofti*, *Brugia malayi*, and *Loa loa*. Rarely, patients with onchocerciasis who are also heavily infected with *L. loa* develop a serious or even fatal encephalopathy either spontaneously or following treatment with an effective microfilaricide. For these patients, the following adverse experiences have also been reported: back pain, conjunctival hemorrhage, dyspnea, urinary and/or fecal incontinence, difficulty in standing and/or walking, mental status changes, confusion, lethargy, stupor, or coma. This syndrome has been seen very rarely following the use of ivermectin; a cause-and-effect relationship has not been established. For individuals who warrant treatment with ivermectin for any reason and have had significant exposure to *L. loa*-endemic areas of West or Central Africa, pretreatment assessment for loiasis and careful posttreatment follow-up should be implemented.

Lumefantrine/Artemether (Coartem, Riamet) (Novartis)

Preparation

The drug is supplied as tablets: 20 mg to 120 mg (scored). This is an antimalarial drug, used for chloroquine-resistant *Plasmodium falciparum*.

Administration

This drug should be taken with food. Do not co-administer with CYP3A4 inducers (rifampin, carbamazepine, phenytoin, St. John's wort). It is not approved for prevention of malaria or for patients with severe or complicated *P. falciparum* malaria. It may prolong the QT interval; avoid with congenital QT interval prolongation (e.g., long QT syndrome) or any other clinical condition known to prolong the QT interval (e.g., history of symptomatic cardiac arrhythmias, clinically relevant bradycardia, severe cardiac disease), family history of congenital QT interval prolongation or sudden death, or known disturbances of electrolyte balance (e.g., hypokalemia, hypomagnesemia). Closely monitor patients who remain averse to food during treatment as risk of recrudescence may be greater; if recrudescent *P. falciparum* infection develops after treatment, treat the patient with a different antimalarial drug. Administer with caution in cases of severe hepatic/renal impairment.

Toxicity

The drug can cause headache, dizziness, anorexia, asthenia, pyrexia, chills, fatigue, arthralgia, myalgia, nausea and/or vomiting, abdominal pain, splenomegaly, hepatomegaly, and cough.

Comment

Artemether: Artemisinin derivative; antimalarial activity attributed to endoperoxide moiety. Lumefantrine: Antimalarial agent; not established. The drug is thought to inhibit the formation of β-hematin by forming a complex with hemin. Both artemether and lumefantrine inhibit nucleic acid and protein synthesis.

Malathion (Ovide) (Taro Pharmaceuticals)

Preparation

Ovide is supplied as a 0.5% aqueous emulsion and is used to treat human louse infestations, killing both lice and nits. Malathion is an organophosphate agent which acts as a pediculicide by inhibiting cholinesterase activity *in vivo*.

Administration

The drug is applied as a lotion to the skin or hair. It is placed on dry hair in a sufficient amount to thoroughly wet the hair. The hair is then allowed to dry naturally and shampooed after 8 to 12 h. It is then rinsed, and a fine comb is used to remove dead lice and nits. If lice are still present 7 to 9 days later, the drug should be reapplied.

Toxicity

Malathion may cause acute renal failure. Because the potential for transdermal absorption of malathion from Ovide lotion is not known at this time, strict adherence to the dosing instructions regarding its use in children, method of application, duration of exposure, and frequency of application is required.

Contraindications

The drug should not be given to individuals exposed to organophosphorous compounds or to infants or neonates. It may cause skin irritation. Because animal reproduction studies are not always predictive of human responses, this drug should be used (or handled) during pregnancy only if clearly needed. The safety and effectiveness of Ovide lotion in children younger than 6 years has not been established in well-controlled trials.

Mebendazole (Generics)

Preparation

The drug is supplied as 100-mg chewable tablets; it is a benzimidazole, broad-spectrum agent used to treat nematode infections. Mebendazole is indicated for the treatment of *Enterobius vermicularis* (pinworm), *Trichuris trichiura* (whipworm), *Ascaris lumbricoides* (common roundworm), *Ancylostoma duodenale* (common hookworm), and *Necator americanus* (American hookworm) in single or mixed infections. In humans, approximately 2% of the administered drug is excreted in urine and the remainder is excreted in the feces as unchanged drug or a primary metabolite. Mebendazole inhibits formation of worms' microtubules and causes worms' glucose depletion.

Administration

Tablets are chewable, but it may be necessary to pulverize them and mix them with food for administration to young children. The drug inhibits the formation of nematode microtubules and causes glucose depletion in the worms. Periodic assessment of the hematopoietic and hepatic system is advisable during prolonged therapy.

Toxicity

Abdominal pain and diarrhea occasionally occur, particularly with the expulsion of a large number of worms in children. Rash, urticaria, and angioedema have been observed on rare occasions. Very rare reports of convulsions have been documented.

Contraindications

The drug is teratogenic in rats, but the incidence of abortion and malformations in humans does not exceed the rate in the general population. The drug, however, is not recommended in pregnancy, particularly during the first trimester, unless the benefit justifies the potential risk to the fetus. It is also not recommended during lactation. In patients with severe hepatic disease, the drug should be used with caution. There is no evidence that mebendazole, even at high doses, is effective for treating hydatid disease. There have been rare reports of neutropenia and agranulocytosis when the drug was taken for prolonged periods and at dosages substantially above those recommended.

Mefloquine Hydrochloride (Generics)

Preparation

The drug is supplied as 250-mg tablets equivalent to 228 g of the free base; it is a synthetic 4-quinoline methanol compound structurally related to quinine. The bioavailability of the tablet formation compared with an oral solution was over 85%. The presence of food significantly enhances the rate and extent of absorption, leading to about a 40% increase in bioavailability. Mefloquine hydrochloride is used for the treatment of mild to moderate acute malaria caused by susceptible strains of *Plasmodium falciparum* and/or *P. vivax*. It is also used for prophylaxis of *P. falciparum* and *P. vivax* malaria infections, including prophylaxis of chloroquine-resistant strains of *P. falciparum*.

Administration

Tablets should not be ingested on an empty stomach and should be taken with at least 8 oz of water.

Toxicity

The most frequent side effects include nausea, vomiting, dizziness, diarrhea, abdominal pain, and headache. Severe seizures and delirium have been reported occasionally in roughly 1 in 200 to 1 in 1,300 patients treated for acute *P. falciparum* malaria. The drug can also potentiate dysrhythmias in patients taking beta blockers. Periodic evaluation of hepatic function should be performed during prolonged prophylaxis.

Contraindications

Use of mefloquine is contraindicated in patients with a known hypersensitivity to mefloquine or related compounds (e.g., quinine and quinidine). The drug should not be prescribed for prophylaxis in patients with active depression, a recent history of depression, generalized anxiety disorder, psychosis, schizophrenia, or other major psychiatric disorders, or in those with a history of convulsions. In patients with epilepsy, the drug may increase the risk of convulsions. The drug should be used with caution in patients with severe hepatic or renal dysfunction. During prophylactic use, if psychiatric symptoms such as acute anxiety, depression, restlessness, or confusion occur, these may be considered prodromal to a more serious event. In these cases, the drug must be withdrawn and an alternative medication should be substituted.

In case of life-threatening, serious, or overwhelming malaria infections with P. falciparum*, patients should be treated with an i.v. antimalarial drug. Following completion of i.v. treatment, mefloquine may be given to complete the course of therapy.*

Comment

Although its exact mechanism of action is not known, mefloquine is an antimalarial agent which acts as a blood schizonticide. The drug is thought to act by interfering with the digestion of hemoglobin during the erythrocytic stages of the life cycle. The drug has no activity against tissue schizonts or gametocytes. Strains of *P. falciparum* with decreased susceptibility to mefloquine can be selected *in vitro* or *in vivo*. Resistance of *P. falciparum* to mefloquine has been detected in areas of multidrug resistance in Southeast Asia. Increased incidences of resistance have also been reported in other parts of the world.

Strains of *P. falciparum* resistant to mefloquine have been detected. Mefloquine is indicated for the treatment of mild to moderate acute malaria caused by mefloquine-susceptible strains of *P. falciparum* (both chloroquine-susceptible and -resistant strains) or by *P. vivax*. However, patients with *P. vivax* malaria are at risk of relapse since mefloquine does not eliminate the exoerythrocytic parasites in the liver. Mefloquine is also indicated for the prophylaxis of *P. falciparum* and *P. vivax* malaria infections, including those caused by chloroquine-resistant strains of *P. falciparum*.

Mefloquine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women of childbearing potential who are traveling to areas where malaria is endemic should be warned against becoming pregnant. Women of childbearing potential should also be advised to practice contraception during malaria prophylaxis with mefloquine and for up to 3 months thereafter. However, in the case of unplanned pregnancy, malaria chemoprophylaxis with mefloquine is not considered an indication for pregnancy termination. Because of the potential for serious adverse reactions in nursing infants from mefloquine, a decision should be made whether to discontinue treatment, taking into account the importance of the drug to the mother.

Melarsoprol (Mel-B) (CDC)

Preparation

Melarsoprol is supplied through the CDC Parasitic Disease Drug Service as a 3.6% solution in propylene glycol. It is used to treat African trypanosomiasis. The drug should be kept refrigerated. It is to be administered only in the hospital by or under the immediate supervision of a physician.

Administration

The drug should be given in propylene glycol as a slow i.v. infusion in the hospital. Care must be taken to avoid extravasation.

Toxicity

Abdominal pain, nausea, vomiting, and diarrhea are common. Reactive encephalopathy is not uncommon and is more likely to occur in debilitated patients and when high doses are used. The patient may also exhibit confusion, speech difficulties, restlessness, and sleep disorders. Myocardial damage, albuminuria, and hypertension have also been reported. A rare reaction is shock and subsequent death. Due to toxicity, the drug is usually reserved for the late stages of disease involving the central nervous system.

Rare side effects may include black, tarry stools; blood in urine or stools; changes in skin color of face; fainting; fast or irregular breathing; pinpoint red spots on skin; puffiness or swelling of the eyelids or around the eyes; shortness of breath; sores, ulcers, or white spots on lips or in mouth; sore throat; swollen and/or painful glands; tightness in chest; trouble in breathing; unusual bleeding or bruising; unusual tiredness or weakness; or wheezing.

Contraindications

Patients with impaired hepatic or renal function should not receive melarsoprol. Severe hemolytic reactions can occur in patients with G6PDH deficiency, and erythema nodosum may be induced in patients with leprosy. The drug should not be given during influenza epidemics. It may increase the chance of side effects in patients with fever and may increase the chance of side effects affecting the blood in patients with G6PDH deficiency.

Comment

Melarsoprol, an investigational drug, is an arsenical compound used for treating African trypanosomiasis (*Trypanosoma brucei gambiense*, *T. brucei rhodesiense*) with neurologic involvement or early African trypanosomiasis that is resistant to suramin or pentamidine. The drug apparently interferes with parasite energy generation and trophozoite multiplication.

Metronidazole (Flagyl, IV Flagyl) (Searle/Pfizer, Baxter)

Preparation

Flagyl is supplied as 375-mg tablets for oral administration and as 750-mg extended-release tablets (Flagyl ER); it is used in the treatment of amebiasis, giardiasis, trichomoniasis, and some helminth infections. It is also available in single-dose vials containing 500 mg of lyophilized powder or in 100-ml plastic containers containing a solution of 500 mg/100 ml or isotonic-buffered solution for parenteral injection. If metronidazole is being prescribed for trichomoniasis, sexual partners are often treated at the same time, even if they are asymptomatic.

Administration

Flagyl is usually given orally. When given i.v., it should be administered slowly as an intermittent or continuous infusion. Other drugs should not be added to the i.v. line during the infusion. Care must be taken to follow instructions for reconstitution of the solution, because neutralization with bicarbonate is necessary to bring the pH from 0.5–2 up to the neutral range. Administration i.v. can be used in cases of invasive amebiasis.

Toxicity

Reactions to therapy are common, especially when the drug is given in doses appropriate for treating amebiasis. The more common side effects include anorexia, nausea, vomiting, diarrhea, epigastric distress, and abdominal cramps. A metallic taste in the mouth is often reported. Headache, dizziness, and vertigo may also occur. Ataxia, paresthesias, insomnia, and irritability may also occur. A flattening of the T waves on an electrocardiogram may be evident, and rashes and urticaria have been reported. A moderate leukopenia occasionally occurs, but levels return to normal after completion of therapy. Oral and vaginal overgrowth of yeast cells may occur. In patients receiving high doses, dark urine has occurred; this finding may be due to a metabolite of the drug. Rare reactions include blood dyscrasias, dysuria, incontinence, cystitis, dyspareunia, and fever.

Contraindications

The drug should not be given to patients with a history of blood dyscrasia, active central nervous system disease, and/or known sensitivity to metronidazole. Doses should be reduced in patients with severe liver disease. The drug should not be used in pregnancy, especially in the first trimester, unless the benefits of treatment outweigh the potential hazard to the fetus. Consumption of alcohol should be avoided during the first days of treatment and for at least 3 days after the end of the treatment because severe vomiting, abdominal cramps, and flushing may occur. Other alcohol-containing preparations (for example, elixirs, cough syrups, and tonics) may also cause problems. These problems may last for at least a day after the drug has been discontinued. Leukocyte counts with differential blood counts are recommended before and after therapy. A good alternate for use during pregnancy is paromomycin.

Miltefosine (Impavido, Miltex) (Paladin, Canada) (CDC)

Preparation

The drug is given orally at 30 mg/kg twice a day; it has activity against *Leishmania* spp. It is not available within the United States. In India, it has been given at 2.5 mg/kg/day for 4 weeks, with good results for visceral leishmaniasis. Good activity has also been documented against *L. viannia panamensis*, with less activity against *L. mexicana mexicana* and even lower for *L. viannia braziliensis*. It is also used to treat granulomatous amebic encephalitis caused by *Acanthamoeba* spp.

Administration

The drug is given in two divided doses or a single dose orally for 28 days. It is available as 50-mg and 10-mg capsules.

Toxicity

Most patients tolerate therapy with few or no side effects. Nausea, vomiting, and diarrhea have been reported, and there have been isolated reports of dose-related motion sickness. Some patients have reversible hepatotoxicity and nephrotoxicity. Urea and creatinine levels usually return to normal by the end of the second week of therapy. Gastrointestinal symptoms could be serious in severely ill patients who are malnourished and dehydrated.

Contraindications

The drug should not be used during pregnancy or lactation.

Comment

The mechanism is not well defined, but apparently the drug interferes with cell-signaling pathways and membrane synthesis. It also induces apoptotic cell death. It is active against the extracellular promastigote and the intracellular amastigote forms of *Leishmania* spp.

Niclosamide (Yomesan, Niclocide) (Bayer, Germany)

Preparation

Niclosamide is available in 500-mg chewable tablets (chlorosalicylamide), which is effective against infections with tapeworms (*Taenia* spp., etc). The drug is no longer available in the United States. Its use is limited, and praziquantel is now more commonly used.

Administration

The drug is given orally; however, to prevent stomach upset, it is best taken after a light meal (for example, breakfast). Niclosamide tablets should be thoroughly chewed or crushed and then swallowed with a small amount of water. If this medicine is being given to a young child, the tablets should be crushed to a fine powder and mixed with a small amount of water to form a paste.

Toxicity

Most patients tolerate therapy with few or no side effects. Nausea, vomiting, and abdominal pain occur rarely, and there have been isolated reports of fever, dizziness, urticaria, irritability, headache, and edema of the arms.

Contraindications

Niclosamide should not be given to patients shown to be hypersensitive to the drug. Insufficient data are available for an assessment of the hazards of use of this drug during pregnancy. It is not recommended for pregnant women and children younger than 2 years.

Nifurtimox (Lampit) (Bayer HealthCare) (CDC)

Preparation

Nifurtimox is a nitrofuran and is supplied as 100-mg tablets through the CDC Parasitic Disease Drug Service; it is used in the treatment of acute Chagas' disease.

Administration

The drug is given orally. Tolerance for the drug decreases with age, and children generally can tolerate higher doses over a longer period than can adults.

Toxicity

Gastrointestinal symptoms are common and may include anorexia, weight loss, abdominal pain, nausea, and vomiting. Neuropsychiatric symptoms, including convulsions, headache, drowsiness, nervousness, vertigo, paresthesia, disorientation, and memory loss, are less common but may necessitate termination of therapy.

Contraindications

The drug should not be used in pregnant women or in children younger than 1 year. Alcohol, antibiotics, and digitalis may potentiate the side effects. It should be used with caution in elderly patients with a history of neurologic or psychiatric diseases or allergic skin reactions.

Comment

Nifurtimox, an investigational drug, is a nitrofurfurylidene derivative. Its action is apparently related to its ability to cause the production of toxic reduced products of oxygen such as superoxide, hydrogen peroxide, and hydroxyl radicals. Accumulation of these products within the parasites leads to membrane damage, enzyme inactivation, and possible inhibition of protein synthesis. Nifurtimox is used to treat acute, subacute, or early chronic Chagas' disease. Although only two cases of indigenous Chagas' disease have been reported from the United States, infections may be seen in laboratory workers, immigrants, and U.S. citizens returning from Latin America, such as Peace Corps volunteers, military personnel, and missionaries. Drinking alcoholic beverages while taking this medicine may cause some unwanted effects. Other alcohol-containing preparations (for example, elixirs, cough syrups, and tonics) may also cause problems. Therefore, the patient should not drink alcoholic beverages or ingest other alcohol-containing preparations while taking nifurtimox.

Nitazoxanide (Alinia) (Romark)

Preparation

Nitazoxanide is a relatively new synthetic drug with good efficacy against a number of intestinal protozoa and helminths. Alinia tablets contain 500 mg of nitazoxanide, and Alinia for oral suspension, after reconstitution, contains 100 mg nitazoxanide per 5 ml. Nitazoxanide and its metabolite, tizoxanide, are active *in vitro* in inhibiting the growth of sporozoites and oocysts of *Cryptosporidium* spp. and trophozoites of *Giardia lamblia*. Nitazoxanide may also be effective in the treatment of intestinal microsporidiosis with *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, as well as ocular infections with *Vittaforma corneae* (see dosage information under *Giardia lamblia*). However, nitazoxanide was not shown to be superior to placebo for treatment of diarrhea caused by *Cryptosporidium parvum* in HIV-infected or immunodeficient patients.

Administration

The drug should be taken orally with food. Diabetic patients and their caregivers should be aware that the oral suspension contains 1.48 g of sucrose per 5 ml. Alinia for oral suspension should be used for pediatric patients younger than 11 years.

Toxicity

The most common adverse events reported were abdominal pain, diarrhea, headache, and nausea. Other reported side effects included weakness, fever, pain, chills, flu syndrome, dizziness, somnolence, insomnia, rash, and pruritus.

Contraindications

The pharmacokinetics of nitazoxanide in patients with compromised renal or hepatic function has not been studied. Therefore, nitazoxanide must be administered with caution to patients with hepatic and/or biliary disease, renal disease, and combined renal and hepatic disease. Nitazoxanide is highly bound to plasma protein; competition for binding sites could occur with other drugs such as warfarin. Based on limited safety data, this drug should not be used during pregnancy or lactation.

Comment

The antiprotozoal activity of nitazoxanide is thought to be due to interference with the pyruvate ferredoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction, which is essential to anaerobic energy metabolism. Studies have shown that the PFOR enzyme from *G. lamblia* directly reduces nitazoxanide by transfer of electrons in the absence of ferredoxin. The DNA-derived PFOR protein sequence of *Cryptosporidium* spp. appears to be similar to that of *G. lamblia*. Interference with the PFOR enzyme-dependent electron transfer reaction may not be the only pathway by which nitazoxanide exhibits antiprotozoal activity.

Nitazoxanide therapy has also been given for intestinal microsporidiosis at a dose of 1,000 mg twice a day for 60 consecutive days. Three posttreatment fecal examinations, including PCR, conducted over 2 months following the end of the treatment with nitazoxanide did not reveal any microsporidial spores, and the patient continued normal bowel movements. Nitazoxanide has also been reported to be effective in cell culture against *E. intestinalis* and *V. corneae*.

Paromomycin (Generics) (Sun Pharma)

Preparation

Humatin is supplied as 250-mg capsules and a syrup with 125 mg per 5 ml; a broad-spectrum antibiotic, it is used to treat various intestinal protozoal infections, particularly acute and chronic intestinal amebiasis. The drug is poorly absorbed after oral administration, with almost 100% of the drug being recoverable in the stool. The drug is also prepared as a topical (*Leshcutan*) for the treatment of mucocutaneous leishmaniasis.

Administration

The drug is given orally with a full glass of water and should be taken with food.

Toxicity

Nausea, vomiting, abdominal cramps, and diarrhea have been reported, particularly in patients taking more than 3 g/day. Dizziness has also been reported. Assess for history of hypersensitivity to the drug, intestinal obstruction, and ulcerative lesions of the bowel.

Contraindications

The drug should not be used in patients with impaired renal function, intestinal obstruction, or ulcerative bowel lesions. Paromomycin and other aminoglycosides should be avoided in patients with myasthenia gravis. The drug should not be used during pregnancy or lactation.

Pentamidine Isethionate (Pentam 300, Nebupent) (APP Pharmaceuticals)

Preparation

Pentamidine isethionate is supplied as 300-mg vials for i.v. or i.m. administration. For inhalation, the vial contents are dissolved in sterile water and the inhalation solution is placed in a Respirgard II nebulizer. Saline solution must not be used to reconstitute the drug. Pentamidine isethionate is a toxic but highly effective drug used to treat the early stages of both types of African trypanosomiasis (*Trypanosoma brucei gambiense* and *T. brucei rhodesiense*). It does not cross the blood-brain barrier and is not effective in the treatment of the neurologic phases of the disease. It is also used to treat leishmaniasis and *Pneumocystis jirovecii* (now classified with the fungi) infection. The drug is also used to treat free-living amebae infections, such as granulomatous amebic encephalitis caused by *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Sappinia* sp.

Administration

Vial contents should be dissolved in sterile water for i.m. injection or in sterile water followed by sterile 5% glucose for i.v. administration. Pentamidine for inhalation should be administered for approximately 35 to 40 min at a rate of 5 to 7 liters/min.

Toxicity

Common side effects include hypotension, tachycardia, nausea, vomiting, and a metallic taste that occurs immediately after injection but subsides in 10 to 30 min. Hypoglycemia is occasionally seen from days 5 to 7 of therapy. Acute renal failure has occurred during the course of therapy; however, it is usually reversible in patients with normal renal function before therapy. Mild anemia, elevated liver transaminase levels, and leukopenia have been reported. Local pain and inflammation of the injection site are common, and sterile abscesses occasionally occur. Hypotension and profound shock have been reported after a single dose of the drug, and resuscitation equipment should always be readily available in the setting in which drug therapy is given. Occasional seizures and hallucinations have also been reported. Inhaled pentamidine can cause cough, bronchospasm, fatigue, bad taste in the mouth, shortness of breath, decreased appetite, dizziness, and rash. Pentamidine has also been associated with Stevens-Johnson syndrome.

Contraindications

Because pentamidine is generally used in life-threatening situations, there are no absolute contraindications to therapy. Careful observation of the patient is necessary because of the common side effects and severe drug reactions that may occur. Impaired renal function is frequent. Aerosol pentamidine should not be given with other drugs or given to patients with a history of anaphylactic reactions to inhaled or parenteral pentamidine. Some patients may develop a sudden, severe decrease in blood pressure after a dose of pentamidine. Pentamidine can decrease the white blood cell count, increasing the chance that the patient will contract certain infections. It can also lower the number of platelets, which are necessary for proper blood clotting. Both low and high blood sugar levels have also been reported. Unless the benefits outweigh the potential risks, this drug should not be used during pregnancy.

Comment

Although its mode of action remains unclear, its activity may be different for different parasites. Pentamidine (Lomidine) is used only for the early phase of the disease, since it does not cross the blood-brain barrier. It is active against *T. gambiense*, but it should not be used against *T. rhodesiense*, since primary resistance to it has been found in some areas. It should also not be used in areas where pentamidine has been widely employed for chemoprophylaxis, such as in the Democratic Republic of the Congo.

Permethrin (Nix [Insight Pharmaceuticals], Elimite [Prestium Pharma])

Preparation

Permethrin is supplied as a 1 or 5% cream. It is used to treat scabies (a skin infestation) and lice infestations of the head, body, and pubic area ("crabs"). Permethrin does not prevent these infestations.

Administration

The drug is available as a 1% (Nix) or 5% (Elimite) cream applied directly to hair or skin to treat lice or scabies, respectively.

If using the liquid, follow these steps: shampoo the hair using regular shampoo; thoroughly rinse and towel dry hair and scalp; allow hair to air dry for a few minutes; do not stand (or sit) in a shower or bathtub; lean over a sink to apply permethrin to the hair; shake the bottle of liquid well; thoroughly wet hair and scalp with the liquid. Cover the areas behind the ears and the back of the neck; keep permethrin on the hair for 10 minutes before rinsing it off thoroughly with water; dry hair with a clean towel; comb hair with a fine-tooth comb to remove nits (lice eggs and larvae); wash hands to remove the medication.

If using the cream, follow these steps: wash entire body or take a shower; thoroughly massage the cream into the skin over the entire body, from head to toes; leave the cream on for 8 to 14 h; wash the cream off after 8 to 14 h by taking another shower; if using product for head lice, wash combs and brushes with permethrin liquid and rinse them thoroughly with water to remove the drug.

Toxicity

Side effects are rare and include occasional burning, stinging, and numbness; increased pruritus; pain; edema; erythema; and rash.

Contraindications

The cream or lotion should not be applied near the eyes or mucous membranes. The drug may cause progressive motor neuron disease.

Polyhexamethylene Biguanide (Baquacil) (Zeneca)

Preparation

Baquacil is supplied as a 20% solution, which must be diluted with sterile saline to 0.02% for ophthalmic application. It is used to treat keratitis caused by *Acanthamoeba* spp. It is important to be cautious when using corticosteroids in patients with a previous history of *Acanthamoeba* keratitis (AK), even in the relatively distant past. Patients with AK should be warned regarding the risks of recurrence following presumed resolution and the need for retreatment.

Administration

Topical application is recommended at hourly intervals until clinical improvement is noted. Polyhexamethylene-biguanide is available through Leiter's Park Avenue Pharmacy, San Jose, CA (800-292-6773; www.leiterrx.com).

Toxicity

No information is available.

Contraindications

No information is available.

Comment

While this therapy inactivates the trophozoites and cysts in *Acanthamoeba* keratitis in the majority of patients (approximately 90%), there have been notable failures, particularly when patients present late with deep stromal infection. Since no single drug can be assumed to be effective against all amebae, a potential complication is the risk of activation of dormant cysts which form *in situ* in *Acanthamoeba* and *Balamuthia* infections and which can lead to relapse following apparently effective treatment. This is particularly true for *Acanthamoeba* keratitis, a non-opportunistic infection of the cornea, which responds well to treatment with chlorhexidine gluconate and polyhexamethylene biguanide in combination with propamidine isethionate (Brolene), hexamidine (Desomodine), or neomycin. In a large series of *Acanthamoeba* keratitis patients with a positive microbiologic diagnosis at presentation, nearly 5% developed recurrent episodes of corneal and scleral inflammation with viable *Acanthamoeba* in the cornea despite prolonged treatment with biguanides and/or diamidines.

Praziquantel (Biltricide) (Bayer)

Preparation

Praziquantel is supplied as 150-, 500-, and 600-mg scored tablets. It is a pyrazinoisoquinoline derivative and is a synthetic heterocyclic broad-spectrum anthelmintic agent effective against all schistosome species infecting humans, as well as most other trematodes and adult cestodes. However, it is not useful for treatment of nematode infections and is not effective in treating *Fasciola hepatica*. It has also been used in the treatment of cysticercosis.

Administration

Praziquantel is preferably taken during a meal, particularly one with a high carbohydrate content. After oral administration, over 80% of the drug is absorbed.

Toxicity

In general, the drug is well tolerated; side effects are usually mild. Reactions include malaise, headaches, dizziness, abdominal discomfort, nausea, vomiting, fever, and rarely urticaria. When it is used in high doses, as in the treatment of central nervous system cysticercosis, reactions that have been attributed to the death of the larvae can occur. These reactions include headache, peripheral neuropathy, seizures, fever, hypotension, and shock. Anaphylaxis has also been reported. Transient hyperglycemia lasting several months after therapy has also been reported. Patients are generally hospitalized during the initiation of therapy for cysticercosis because of the potential for serious reactions such as seizures and other neurologic sequelae related to an inflammatory response.

Contraindications

The drug induces drowsiness and dizziness and so should be used with caution in patients whose vocation requires mental alertness. If the drug is given to patients with ocular cysticercosis, parasite destruction may cause irreparable eye damage. Although studies involving high doses of praziquantel in pregnant animals have failed to show any harm to the fetus, there are no controlled studies of humans. The use of praziquantel in pregnant women and in children younger than 4 years has not been well studied, and both the risks and the benefits must be considered. Praziquantel does appear in human milk, and mothers should not breastfeed during treatment and for 72 h following treatment. Concomitant administration of rifampin should be avoided.

Comment

Praziquantel causes ultrastructural changes in the helminth tegument, causing increased permeability to calcium ions. It works by causing severe spasms and paralysis of the worms' muscles. Some kinds of worms are then passed in the stool. However, the patient may not notice them since they are sometimes completely destroyed in the intestine.

Primaquine Phosphate (Sanofi-Aventis)

Preparation

The drug is supplied in tablets containing 26.3 mg of primaquine phosphate, equivalent to 15 mg of salt. It is an antimalarial agent especially effective against *Plasmodium vivax* exoerythrocytic forms, terminating relapsing vivax malaria; it is usually administered with chloroquine. It is also effective against gametocytes of *P. falciparum*.

Administration

Tablets have a bitter taste and may be crushed and added to a sweet liquid or fruit to make them more palatable.

Toxicity

Patients may experience abdominal pain, cramps, or epigastric distress. High doses may result in methemoglobinemia and cyanosis. Granulocytopenia and granulocytosis, hypertension, and arrhythmia are rare. Hemolysis occurs in individuals with G6PDH deficiency.

Contraindications

The drug should not be used in patients with depressed blood counts resulting from other illnesses or concurrently with drugs, such as quinacrine, that have the potential to cause hemolysis or bone marrow depression. Because primaquine phosphate can cause hemolytic anemia, patients should be screened for G6PDH deficiency, which is most common in African, Asian, and Mediterranean people. Administration of other bone marrow depressants as hemolytic agents should be avoided. The drug should not be prescribed during pregnancy.

Propamidine Isethionate (Brolene) (Aventis, Canada)

Preparation

Brolene is provided as a 0.1% solution. It is a local anti-infective agent that is used to treat keratitis caused by *Acanthamoeba* spp., but it is not available in the United States.

Administration

Brolene is administered topically each hour for the first few days and every few hours thereafter for a minimum course of 1 month.

Toxicity

The drug may cause keratopathy; this condition is reversible but may be confused with persistent or recurrent infection.

Contraindications

No information is available.

Comment

Treatment of *Acanthamoeba* keratitis is difficult due to the organism's ability to encyst. Effective medications include topical polyhexamethylene biguanide, propamidine isethionate (Brolene), chlorhexidine digluconate 0.02%, polymixin B, neomycin, and clortrimazole 1%. In one series of 10 patients, the combination of Brolene and polyhexamethylene biguanide successfully cured all cases of *Acanthamoeba* keratitis. Cautious introduction of topical steroids along with antiamebic therapy helped resolve the inflammation and provided symptomatic relief.

Pyrantel Pamoate (Pin-X, Reese's Pinworm Medicine) (Quartz Specialty Pharmaceuticals, Reese)

Preparation

Liquid medication is supplied in 60-ml bottles containing 50 mg of pyrantel base per ml or as tablets containing 125 mg of base and is used to treat a number of different nematode infections. However, it is associated with more side effects and is being replaced with the benzimidazoles, which are more effective.

Administration

Pyrantel pamoate may be taken with food, milk, or juice.

Toxicity

Reactions are not frequently encountered, but when they do occur, they involve primarily the gastrointestinal system. They include anorexia, nausea, vomiting, abdominal pain or cramps, diarrhea and tenesmus, and transient elevations of the transaminase enzyme levels.

Contraindications

Studies of pregnant animals have failed to show any harm to the fetus, but there is no experience with pregnant humans. There are few published data on the use of the drug in children younger than 2 years. The risks and benefits of treatment in these patients should be weighed. The drug should be used with caution in patients with liver dysfunction. Its anthelmintic effect may be antagonized by piperazine.

Comment

The drug is a neuromuscular blocking agent, resulting in spastic paralysis of the adult worms. Excellent results are obtained when treating patients with ascariasis, hookworm, and pinworm infections. While there is some activity against *Trichostrongylus,* the drug is not active against *Trichuris trichiura.*

Pyrethrin with Piperonyl Butoxide (Rid) (Bayer, Others)

Preparation

Rid is an insecticide mixture of permethrin and piperonyl butoxide, which is used as a synergist and also to treat human louse infestations.

Administration

Rid is applied topically as a shampoo to the affected area after wetting. After application, it should be left for 10 min and then rinsed thoroughly. Hair should be combed with a fine-tooth comb to remove dead lice and nits.

Toxicity

Rid may cause allergic reactions in individuals allergic to ragweed.

Contraindications

The drug should not be used near eyes or mucous membranes.

Pyrimethamine (Daraprim) (Amedra)

Preparation

Daraprim is supplied as 25-mg tablets; it is a potent folic acid antagonist used as an antimalarial agent and in treating toxoplasmosis and several other parasitic infections. Dosages for treating toxoplasmosis approach the toxic level. Daraprim should be kept away from children and infants since they are extremely susceptible to adverse effects from an overdose and deaths have been reported.

Administration

The drug may be pulverized and mixed with sweet liquid or fruit juice. Concurrent administration of folinic acid is strongly recommended for all patients. Daraprim is indicated for the treatment of toxoplasmosis when used conjointly with a sulfonamide, providing synergy with this combination approach. Although Daraprim is used to treat acute malaria, it should not be used alone for this purpose. It is also used for chemoprophylaxis of malaria; however, there is widespread resistance throughout the world.

Toxicity

Gastrointestinal complaints (anorexia, nausea, vomiting, diarrhea, and abdominal pain and tenderness) are not uncommon. Occasional elevation of the glutamic-oxaloacetic transaminase level in serum has been reported, as have skin rashes, headache, dizziness, drowsiness, and insomnia. For patients with toxoplasmosis being treated with high doses, periodic complete blood counts, including platelet counts, are recommended. Folic acid supplementation may be necessary if the counts are depressed. Hypersensitivity reactions, occasionally severe (Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme, and anaphylaxis), can occur, particularly when the drug is administered concomitantly with a sulfonamide.

Contraindications

The drug should not be used in pregnancy, and determination of human chorionic gonadotropin levels is recommended before initiation of treatment for toxoplasmosis. Caution is recommended for patients with seizure disorders and impaired hepatic or renal function. Daraprim is also contraindicated for patients with documented megaloblastic anemia due to folate deficiency.

Comment

Pyrimethamine acts against the asexual erythrocytic stage of the *Plasmodium* spp. by inhibiting the enzyme dihydrofolate reductase. The drug does not destroy gametocytes but instead arrests sporogony in the mosquito. Although the drug is active against *P. falciparum*, resistance occurs fairly rapidly and limits its use as a single agent. Therefore, the drug is often combined with a sulfonamide or sulfone, which provides sequential, synergistic inhibition of the folate biosynthesis pathway. This inhibition of folic acid biosynthesis prevents DNA replication in the parasite. Pyrimethamine is also used in combination with sulfadiazine for treating toxoplasmosis.

Quinidine Gluconate (Generics)

Preparation

Quinidine gluconate is supplied as a solution containing 80 mg/ml for injection and is used to treat severe malaria.

Administration

The drug is given i.v. in normal saline by slow infusion; 800 mg is infused in 40 ml of 5% glucose at a rate of 1 ml/min. Avoid grapefruit juice during administration.

Toxicity

Quinidine causes gastrointestinal irritation, with nausea, vomiting, and diarrhea. Hypersensitivity may occur. Other side effects that have been reported include blurred vision, headache, belly pain, and ringing in the ears and temporary loss of hearing. Prolongation of the QT interval as indicated by an electrocardiogram, ventricular arrhythmia, hypotension, and hypoglycemia can result from the use of this drug at treatment doses. Rapid infusion can cause peripheral vascular collapse and severe hypotension.

Contraindications

The drug may induce hypotension, granulomatous hepatitis, and a lupus-like syndrome. Use should be avoided for patients with myasthenia gravis, acute infections with fever, previous hypersensitivity to quinidine, and cardiac abnormalities.

Quinine Sulfate or Quinine Dihydrochloride (Many Manufacturers)

Preparation

Quinine sulfate is supplied as 3-grain (186-mg) or 6-grain (372-mg) capsules or tablets. Quinine dihydrochloride comes in 2-ml ampoules containing 300 mg/ml (not available in the United States). It is used to treat malaria and is important in areas where i.v. quinine is not available. It is the dextrorotary optical isomer of quinine. It has also been used in the treatment of babesiosis.

Administration

The oral medication is extremely bitter and may be better tolerated if mixed with sweet liquid, fruit, or cereal. For i.v. administration, medication should be diluted in normal saline at 0.5 mg/ml and administered at 1 ml/min every 8 h.

Toxicity

Mild symptoms are common and generally consist of tinnitus, headache, nausea, and disturbed vision. More severe reactions are related to inability to excrete the drug normally and to high doses. These reactions include decreased hearing, blurred vision, photophobia, diplopia, night blindness, scotomata (blind spots), mydriasis (pupil dilation), vomiting, abdominal pain, diarrhea, cutaneous flushing, sweating, rashes, facial angioedema, confusion, apprehension, excitement, delirium, and syncope.

Contraindications

Rare reactions include renal failure, acute hemolysis, hypoprothrombinemia, purpura, agranulocytosis, asthma, and ventricular tachycardia. After one dose, an idiosyncratic hypersensitivity reaction consisting of flushing, pruritus, skin rash, fever, gastric distress, dyspnea, tinnitus, and visual impairment may occur. Massive hemolysis and hemoglobinuria occasionally occur during the treatment of falciparum malaria. Quinine may aggravate the symptoms of myasthenia gravis.

Spiramycin (Rovamycine) (Sanofi-Aventis)

Preparation

Rovamycine is supplied as a tablet or in solution and is a macrolide antibiotic similar to leucomycin. This drug has been tried in the treatment of cryptosporidiosis and toxoplasmosis; it is available only from the manufacturer.

Administration

Spiramycin is given orally on an empty stomach or by i.v. infusion.

Toxicity

Rovamycine can cause gastrointestinal disturbances and occasional allergic reactions. More rare side effects have included bloody stools, chest pain, fever, heartburn, irregular heartbeat, nausea, recurrent fainting, stomach pain and tenderness, vomiting, and yellow eyes or skin.

Contraindications

The drug should not be given to patients with impaired liver function.

Stibogluconate Sodium (Pentostam, Solustibosan) (GlaxoSmithKline) (CDC)

Preparation

Stibogluconate sodium is available through the CDC Parasitic Disease Drug Service as a 33% solution containing 100 mg of pentavalent antimony per ml; it is used to treat leishmaniasis.

Administration

The drug is given intramuscularly or by careful, slow i.v. infusion.

Toxicity

Side effects are common and include nausea, vomiting, severe coughing, and itching. Pneumonitis, arthralgias, myalgias, bradycardia, electrocardiogram changes (T-wave depressions, increased O-T interval, and fusion of the S-T segment and T wave), abnormal liver function, headache, and mild rash also occur. Less common problems are hepatitis, hemolytic anemia, thrombocytopenia, and anaphylaxis associated with urticaria, laryngeal edema, and visceral collagens.

Contraindications

The drug should not be used in patients with myocarditis, hepatitis, liver disease, or concurrent bacterial and viral infections. Treatment should be stopped if there is progressive proteinuria, severe arthralgias, or rash. More severe reactions may include leukopenia, agranulocytosis, and electrocardiographic changes.

Comment

Pentostam (sodium stibogluconate; sodium antimony gluconate) is a pentavalent antimony compound that is used to treat all forms of leishmaniasis (cutaneous, mucocutaneous, and visceral). This drug is a well-established antileishmanial agent that has been in use for some time. It is thought to inhibit glycolysis enzymes within the organisms.

Suramin Sodium (Germanin) (Bayer, Germany) (CDC)

Preparation

Suramin sodium is available through the CDC Parasitic Disease Drug Service as ampoules containing 1.0 g of powder and is a complex derivative of urea used to treat African trypanosomiasis.

Administration

A 10% solution must be prepared and used within 30 min of preparation as a slow i.v. infusion.

Toxicity

Immediate nausea, vomiting, loss of consciousness, and seizure may occur if the full i.v. dose is given to sensitive individuals. A test dose is recommended. Proteinuria is commonly seen during therapy, but it is not a parameter used to monitor therapy unless other renal function studies are abnormal. Fever is also common but is usually low grade. Rashes, localized edema, and pruritus may occur. Blepharitis, conjunctivitis, photophobia, and excessive lacrimation may occur during treatment. Kidney failure, blood dyscrasias (including pancytopenia), shock, and optic atrophy have also been reported.

Contraindications

The drug should be administered under close medical supervision and should not be used in pregnant women or in patients with hepatic or renal insufficiency. It should be used with caution in children younger than 6 years. It should not be used alone to treat African trypanosomiasis of the central nervous system.

Comment

Suramin was introduced in 1920 for the treatment of African trypanosomiasis (sleeping sickness). It is considered the drug of choice for treatment of early African trypanosomiasis with *Trypanosoma brucei rhodesiense* when the central nervous system is not involved. Pentamidine is thought to be more effective than suramin for treating early African trypanosomiasis caused by *T. brucei gambiense*. The mechanism of action of suramin is thought to be through inhibition of enzymes associated with protein synthesis and DNA metabolism.

Thiabendazole (Mintezol) (Merck)

Preparation

Mintezol is supplied as a flavored suspension containing 125 or 250 mg/5 ml, as pediatric drops containing 100 mg/ml, and as 500-mg chewable tablets. Thiabendazole is a benzimidazole that is used to treat a number of nematode infections, including strongyloidiasis, cutaneous and visceral larva migrans, trichinosis, and other intestinal roundworms. However, other drugs have become replacements for thiabendazole.

Administration

The drug is given orally, preferably after a meal; tablets should be chewed before being swallowed.

Toxicity

Common side effects are anorexia, vomiting, and dizziness. Less frequently, diarrhea, epigastric pain, pruritus, drowsiness, lethargy, and headache occur. Side effects include Stevens-Johnson syndrome, tinnitus, visual disturbance, hypotension, perianal rash, transient rises in transaminase enzyme levels, cholestasis, parenchymal liver damage, hyperglycemia, and hematuria. Treatment may cause migration of *Ascaris* organisms to the esophagus, mouth, and nose.

Contraindications

Patients with known hypersensitivity or with renal or hepatic impairment should not use thiabendazole. The drug causes dizziness and drowsiness and should not be used by patients whose vocation requires mental alertness. It is also contraindicated as prophylaxis for pinworm infections; susceptible worm infections should be confirmed by laboratory testing prior to drug use. Due to a lack of safety data, it should be used during pregnancy and lactation only if the potential benefit justifies the potential risk to the fetus.

Comment

Thiabendazole blocks glucose uptake, depletes glucose stores, and interferes with mictotubule aggregates. It also inhibits the fumarate-reductase system of helminths. However, administration of thiabendazole has been associated with side effects, and it has been replaced by other anthelmintic agents.

Tinidazole (Tindamax) (Mission Pharmaceuticals)

Preparation

Tinidazole is supplied as 150-, 200-, 300-, and 500-mg and 1-g tablets or as an oral suspension of 200 mg/ml. This drug is a synthetic nitroimidazole used to treat amebiasis and giardiasis.

Tindamax oral tablets are indicated for the treatment of trichomoniasis caused by *Trichomonas vaginalis* in both female and male patients. The organism should be identified by appropriate diagnostic procedures. Because trichomoniasis is a sexually transmitted disease with potentially serious sequelae, partners of infected patients should be treated simultaneously in order to prevent reinfection. Tindamax oral tablets are indicated for the treatment of giardiasis caused by *Giardia lamblia* in both adults and pediatric patients older than 3 years. Tindamax oral tablets are indicated for the treatment of intestinal amebiasis and amebic liver abscess caused by *Entamoeba histolytica* in both adults and pediatric patients older than 3 years. It is not indicated in the treatment of asymptomatic cyst passage.

Administration

Tinidazole is given orally with or after meals. After oral administration, it is rapidly and completely absorbed. Alcoholic beverages should be avoided while taking Tindamax and for 3 days afterward.

Toxicity

Tinidazole can cause nausea, vomiting, metallic taste, and rash. Convulsive seizures and peripheral neuropathy, the latter characterized mainly by numbness or paresthesia of an extremity, have been reported to occur in patients treated with nitroimidazole drugs including tinidazole and metronidazole. The appearance of abnormal neurologic signs demands the prompt discontinuation of Tindamax therapy. Tinidazole should be administered with caution to patients with central nervous system diseases or those with evidence of or history of blood dyscrasia.

Contraindications

As with other nitroimidazole derivatives, tinidazole may enhance the effect of warfarin and other coumarin anticoagulants, resulting in a prolongation of the prothrombin time. Alcoholic beverages and preparations containing ethanol or propylene glycol should be avoided during tinidazole therapy and for 3 days afterward because abdominal cramps, nausea, vomiting, headaches, and flushing may occur. Psychotic reactions have occurred in alcoholic patients using metronidazole and disulfiram concurrently. Because animal reproduction studies are not always predictive of the human response and because there is some evidence of mutagenic potential, the use of tinidazole during pregnancy requires that the potential benefits of the drug be weighed against the possible risks to both the mother and the fetus. Tinidazole is excreted in breast milk in concentrations similar to those seen in serum. It can be detected in breast milk for up to 72 h following administration. Interruption of breast-feeding is recommended during tinidazole therapy and for 3 days following the last dose.

Comment

The nitro group of tinidazole is reduced by cell extracts of *Trichomonas*. The free nitro radical generated as a result of this reduction may be responsible for the antiprotozoal activity. The mechanism by which tinidazole exhibits activity against *Giardia* and *Entamoeba* species is not known. Approximately 38% of *T. vaginalis* isolates with reduced susceptibility to metronidazole also show reduced susceptibility to tinidazole *in vitro*. The clinical significance of such an effect is not known.

Tinidazole, like metronidazole, may interfere with certain types of determinations of serum chemistry values, such as aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, triglyceride, and hexokinase glucose levels.

Triclabendazole (Egaten) (Novartis)

Preparation

Triclabendazole is used in veterinary practice and is also being used to treat human infections with liver and lung flukes; it the drug of choice for treating infections with *Fasciola hepatica*. The drug is not available in the United States.

Administration

Egaten is given orally after a meal as a single or split dose of 10 mg/kg. A dose of 20 mg/kg is safe and effective for patients with acute fascioliasis when a single dose has failed to cure them.

Toxicity

Triclabendazole can cause abdominal pain; however, most studies reported no side effects. The tolerance of triclabendazole is considered excellent. Side effects include brief episodes of upper abdominal pain and slight fever and some mild and limited disturbances in liver function. These effects may be due to the paralysis and/or death of the flukes, resulting in the release of antigens or toxic products and the partial blockage of the bile ducts. Similar findings have been reported in various preliminary studies: triclabendazole has been reported to decrease parasite motility, but the exact mode of action of this drug is unknown. Some patients may require a second course of therapy.

Contraindications

Use of this drug may cause secondary cholangitis due to worm destruction. Triclabendazole has been reported to be nonmutagenic and nonteratogenic.

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Appendixes

Although common names are frequently used to describe parasitic organisms, these names may represent different parasites in different parts of the world. To eliminate these problems, a binomial system of nomenclature is used in which the scientific name consists of the genus and species. On the basis of life histories and morphology, systems of classification have been developed to indicate the relationships among the various parasite species.

Parasites of humans are classified in five major subdivisions. These include the Protozoa (amebae, flagellates, ciliates, sporozoans, coccidia), the Platyhelminthes or flatworms (cestodes and trematodes), the Acanthocephala or thorny-headed worms, the Nematoda or roundworms, and the Arthropoda (insects, spiders, mites, ticks, etc.). Information on the Protozoa, Nematoda, Platyhelminthes, and Arthropoda is presented in Appendix 1, Table A1.1. This classification scheme is designed to provide some order and meaning to a widely divergent group of organisms. No attempt has been made to include every possible organism, but only those that are considered to be clinically relevant in the context of human parasitology. It is hoped this information will provide some insight into the parasite groupings, thus leading to a better understanding of organism morphology, parasitic infections, and the appropriate clinical diagnostic approach.

Data on distribution of various parasites are found in Tables A1.2 through A1.7; these tables are from Markell EK, Voge M, John DT, *Medical Parasitology*, 6th ed, WB Saunders Co, Philadelphia, PA, 1986. Diagnostic clinical review information is seen in Tables A1.8 through A1.16. Tables A1.17 through A1.20 contain information on stool ordering, collection, the ova and parasite examination, and laboratory test report comments. Table A1.21 provides information on the estimated prevalence of parasitic diseases worldwide.

Flowcharts for various diagnostic kits are seen in Appendix 2. Appendix 3 presents common problems in parasite identification, and Sheets A4.1 through A4.3 are examples of quality control recording sheets. Source information for diagnostic products and teaching materials is provided in Appendix 5. General references, a list of relevant journals and periodicals, and abstracts and bibliographic sources are provided for the reader in Appendix 6.

Appendix 7 contains information that has been published just months prior to final publication of the book. This synopsis of very recent, "late-breaking" publications can assist the reader in having access to the latest information available. I encourage everyone to review this section as they read various chapters throughout the book. Appendix 8 covers the currently approved molecular panels, some of which contain human clinically relevant parasites. Appendix 9 includes the most frequently asked questions for all areas of human parasitology; this section is very comprehensive and represents questions that have been asked by practicing microbiologists and other users of the book. Appendix 10 includes CPT codes that can be used for diagnostic methods for parasitology recovery and diagnosis.

It is hoped that the extensive information contained in the various appendix sections will be valuable to the reader and will serve as a comprehensive reference overall. I'm indebted to all of the readers who provided various types of input to help improve this sixth edition of *Diagnostic Medical Parasitology*.

APPENDIX 1

Information Tables

TABLE A1.1 Classification of human parasites

- **I. Protozoa**
  - 1. **Intestinal amebae**
    - *Entamoeba histolytica*
    - *Entamoeba dispar*[a]
    - *Entamoeba moshkovskii*
    - *Entamoeba bangladeshi*
    - *Entamoeba hartmanni*
    - *Entamoeba coli*
    - *Entamoeba polecki*
    - *Endolimax nana*
    - *Iodamoeba bütschlii*
    - *Blastocystis* spp.
  - 2. **Intestinal flagellates**
    - *Giardia lamblia*[b]
    - *Chilomastix mesnili*
    - *Dientamoeba fragilis*
    - *Pentatrichomonas hominis*
    - *Enteromonas hominis*
    - *Retortamonas intestinalis*
  - 3. **Intestinal ciliates**
    - *Balantidium coli*
  - 4. **Intestinal coccidia and microsporidia**
    - *Cryptosporidium parvum*
    - *Cryptosporidium hominis*
    - *Cryptosporidium* spp.
    - *Cyclospora cayetanensis*
    - *Cystoisospora belli*
    - *Sarcocystis hominis*
    - *Sarcocystis suihominis*
    - Microsporidia
      - *Enterocytozoon bieneusi*
      - *Encephalitozoon* (*Septata*) *intestinalis*
  - 5. **Sporozoa and flagellates from blood and tissue**
    - **Sporozoa (causing malaria and babesiosis)**
      - *Plasmodium vivax*
      - *Plasmodium ovale*
      - *Plasmodium malariae*
      - *Plasmodium falciparum*
      - *Plasmodium knowlesi*
      - *Babesia* spp.
    - **Flagellates (leishmaniae and trypanosomes)**
    - Old World *Leishmania* species
      - *Leishmania* (*Leishmania*) *tropica*
      - *Leishmania* (*Leishmania*) *major*
      - *Leishmania* (*Leishmania*) *aethiopica*
      - *Leishmania* (*Leishmania*) *donovani*
      - *Leishmania* (*Leishmania*) *archibaldi*
      - *Leishmania* (*Leishmania*) *infantum*
    - New World *Leishmania* species
      - *Leishmania* (*Leishmania*) *mexicana*
      - *Leishmania* (*Leishmania*) *amazonensis*
      - *Leishmania* (*Leishmania*) *pifanoi*
      - *Leishmania* (*Leishmania*) *garnhami*
      - *Leishmania* (*Leishmania*) *venezuelensis*
      - *Leishmania* (*Leishmania*) *chagasi*
      - *Leishmania* (*Viannia*) *braziliensis*
      - *Leishmania* (*Viannia*) *colombiensis*
      - *Leishmania* (*Viannia*) *guyanensis*
      - *Leishmania* (*Viannia*) *lainsoni*
      - *Leishmania* (*Viannia*) *naiffi*
      - *Leishmania* (*Viannia*) *panamensis*
      - *Leishmania* (*Viannia*) *peruviana*
      - *Leishmania* (*Viannia*) *shawi*
    - Old World *Trypanosoma* species
      - *Trypanosoma brucei gambiense*

(continued)

TABLE A1.1 *(continued)*

- *Trypanosoma brucei rhodesiense*
- New World *Trypanosoma* species
  - *Trypanosoma cruzi*
  - *Trypanosoma rangeli*

6. **Amebae and flagellates from other body sites**
   - **Amebae**
     - *Naegleria fowleri*
     - *Sappinia diploidea*
     - *Acanthamoeba* spp.
     - *Hartmanella* spp.
     - *Balamuthia mandrillaris*
     - *Entamoeba gingivalis*
   - **Flagellates**
     - *Trichomonas vaginalis*
     - *Trichomonas tenax*
7. **Coccidia, sporozoa, and microsporidia from other body sites**
   - **Coccidia**
     - *Toxoplasma gondii*
   - **Microsporidia**
     - *Nosema ocularum*
     - *Pleistophora* spp.
     - *Pleistophora ronneafiei*
     - *Trachipleistophora hominis*
     - *Trachipleistophora anthropophthera*
     - *Anncaliia vesicularum*
     - *Anncaliia* (*Brachiola*) *algerae*
     - *Anncaliia* (*Brachiola*) *connori*
     - *Encephalitozoon cuniculi*
     - *Encephalitozoon* (*Septata*) *intestinalis*
     - *Encephalitozoon hellem*
     - *Enterocytozoon bieneusi*
     - *Vittaforma corneae* (*Nosema corneum*)
     - *Microsporidium*[c]

II. Nematodes (roundworms)

1. **Intestinal**
   - *Ascaris lumbricoides*
   - *Enterobius vermicularis*
   - *Ancylostoma duodenale*
   - *Necator americanus*
   - *Strongyloides stercoralis*
   - *Strongyloides fuelleborni*
   - *Trichostrongylus colubriformis*
   - *Trichostrongylus orientalis*
   - *Trichostrongylus* spp.
   - *Trichuris trichiura*
   - *Capillaria philippinensis*
2. **Tissue**
   - *Trichinella spiralis*
   - *Trichinella* spp. (*T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*, *T. papuae*, *T. pseudospiralis*, *T. zimbabwensis*)
   - *Toxocara canis* and *Toxocara cati* (visceral and ocular larva migrans)
   - *Ancylostoma braziliense* and *Ancylostoma caninum* (cutaneous larva migrans)
   - *Dracunculus medinensis*
   - *Angiostrongylus cantonensis*
   - *Angiostrongylus costaricensis*
   - *Gnathostoma spinigerum*
   - *Anisakis* spp. (larvae from saltwater fish)
   - *Phocanema* spp. (larvae from saltwater fish)
   - *Contracaecum* spp. (larvae from saltwater fish)
   - *Hysterothylacium*
   - *Porrocaecum* spp.
   - *Capillaria hepatica*
   - *Thelazia* spp.
   - *Ternidens diminutus*
3. **Blood and tissues (filarial worms)**
   - *Wuchereria bancrofti*
   - *Brugia malayi*
   - *Brugia timori*
   - *Loa loa*
   - *Onchocerca volvulus*
   - *Mansonella ozzardi*
   - *Mansonella streptocerca*
   - *Mansonella perstans*
   - *Dirofilaria immitis* (usually found in lung lesions, eyes; in dogs, heartworm)
   - *Dirofilaria* spp. (may be found in subcutaneous nodules)

III. Cestodes (tapeworms)

1. **Intestinal**
   - *Diphyllobothrium latum*
   - *Diplogonoporus* spp.
   - *Dipylidium caninum*
   - *Hymenolepis nana*
   - *Hymenolepis diminuta*
   - *Taenia solium*
   - *Taenia saginata*
   - *Taenia asiatica*
2. **Tissue (larval forms)**
   - *Taenia solium*
   - *Echinococcus granulosus*
   - *Echinococcus multilocularis*
   - *Echinococcus vogeli*
   - *Echinococcus oligarthrus*
   - *Multiceps multiceps*
   - *Spirometra mansonoides*
   - *Diphyllobothrium* spp.

IV. Trematodes (flukes)

1. **Intestinal**
   - *Fasciolopsis buski*
   - *Echinostoma ilocanum*
   - *Echinochasmus perfoliatus*
   - *Heterophyes heterophyes*
   - *Metagonimus yokogawai*
   - *Gastrodiscoides hominis*
   - *Phaneropsolus bonnei*
   - *Prosthodendrium molenkempi*
   - *Spelotrema brevicaeca*
   - *Plagiorchis* spp.
   - *Neodiplostomum seoulense*
2. **Liver and lung**
   - *Clonorchis* (*Opisthorchis*) *sinensis*
   - *Opisthorchis viverrini*
   - *Opisthorchis felineus*
   - *Dicrocoelium dendriticum*

(continued)

TABLE A1.1 Classification of human parasites *(continued)*

- *Fasciola hepatica*
- *Fasciola gigantica*
- *Metorchis conjunctus*
- *Paragonimus westermani*
- *Paragonimus kellicotti*
- *Paragonimus africanus*
- *Paragonimus uterobilateralis*
- *Paragonimus miyazakii*
- *Paragonimus mexicanus*
- *Paragonimus caliensis*

3. **Blood**
    - *Schistosoma mansoni*
    - *Schistosoma haematobium*
    - *Schistosoma japonicum*
    - *Schistosoma intercalatum*
    - *Schistosoma mekongi*
    - *Schistosoma malayi*
    - *Schistosoma mattheei*

V. Pentastomids (tongue worms); *see* Arthropods, below

Tissue (larval forms)
- *Armillifer* spp.
- *Linguatula serrata*
- *Sebekia* spp.

Nasopharyngeal
- *Armillifer* spp.
- *Linguatula serrata*

VI. Acanthocephalans (thorny-headed worms)

Intestine
- *Macracanthorhynchus hirudinaceus*
- *Moniliformis moniliformis*

VII. Arthropods

1. **Arachnida**
    - Scorpions
    - Spiders (black widow, brown recluse)
    - Ticks (*Dermacentor*, *Ixodes*, *Argas*, and *Ornithodoros* spp.)
    - Mites (*Sarcoptes* spp.)
2. **Crustacea**
    - Copepods (*Cyclops* spp.)
    - Crayfish, lobsters, and crabs
3. **Pentastomida**
    - Tongue worms
4. **Diplopoda**
    - Millipedes
5. **Chilopoda**
    - Centipedes
6. **Insecta**
    - Phthiraptera (sucking lice [*Pediculus* and *Phthirus* spp.])
    - Blattaria (cockroaches)
    - Hemiptera (true bugs [*Triatoma* spp.])
    - Coleoptera (beetles)
    - Hymenoptera (bees, wasps, etc.)
    - Lepidoptera (butterflies, caterpillars, moths, etc.)
    - Diptera (flies, mosquitoes, gnats, and midges [*Phlebotomus*, *Aedes*, *Anopheles*, *Glossina*, *Simulium* spp., etc.])
    - Siphonaptera (fleas [*Pulex* and *Xenopsylla* spp., etc.])

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[a] The name *Entamoeba histolytica* is used to designate the true pathogen, while the name *E. dispar* is now being used to designate the nonpathogen. However, unless trophozoites containing ingested red blood cells (*E. histolytica*) are seen, the two organisms cannot be differentiated on the basis of morphology seen in permanent stained smears of fecal specimens. Fecal immunoassays are available for detecting the *Entamoeba histolytica*/*E. dispar* group or for differentiating between the two species. *E. moshkovskii* and *E. bangladeshi* cannot be differentiated from *E. histolytica* with no ingested red blood cells or *E. dispar*.

[b] Although some individuals have changed the species designation for the genus *Giardia* to *G. duodenalis* or *G. intestinalis*, there is no general agreement. Therefore, for this listing, the name *Giardia lamblia* is retained.

[c] The designation *Microsporidium* is not a true genus, but a “catch-all” for organisms that have not been (or may never be) identified to the genus and/or species levels (*Microsporidium ceylonensis*, *Microsporidium africanum*). However, it is now listed as one of the genera of microsporidia infecting humans.

TABLE A1.2 Distribution of selected parasitic infections in the Americas

Category	Distribution[a]				
	North America	Mexico and Central America	Caribbean	Tropical South America	Temperate South America
Protozoa					
Leishmaniasis					
Cutaneous	X•	X	O	X	O
Mucocutaneous	X•	X	O	X	X
Visceral	O	X	O	X	X
Malaria	X•	X	Hispaniola	X	X
Trypanosomiasis (*Trypanosoma cruzi*)	X•	X	O	X	X
Trematodes					
Schistosomiasis (*Schistosoma mansoni*)	O	O	X	X	X
Paragonimiasis	X•	X	O	X	O
Metorchis conjunctus (liver fluke)	X•	O	O	O	O
Cestodes					
Diphyllobothriasis	X	O	O	O	X
Taeniasis (*Taenia solium*)	X•	X	O	X	O
Cysticercosis	X•	X	X	X	X
Hydatid disease	X	X	O	X	X
Nematodes					
Filariasis					
Dirofilariasis	X•	X•	X•	X•	X•
Wuchereria bancrofti	O	X	X	X	O
Onchocerca volvulus	O	X	O	X	O

[a] X, present; O, absent; X•, sporadic or rare occurrence.

TABLE A1.3 Distribution of selected parasitic infections in Europe

Category	Distribution[a]				
	Northwest	Southwest	Central	Eastern	Mediterranean littoral
Protozoa					
Leishmaniasis					
Cutaneous	O	O	O	X	X
Visceral	O	O	O	O	X
Trematodes					
Schistosomiasis (*Schistosoma haematobium*)	O	Portugal	O	O	O
Clonorchiasis, opisthorchiasis	O	O	X	X	O
Cestodes					
Dirofilariasis	N	X	X	X•	X
Diphyllobothriasis	X	O	X	O	O
Hydatid disease	X	X	X	X	X

[a] X, present; X•, sporadic; O, absent; N, no data.

TABLE A1.4 Distribution of selected parasitic infections in Africa

Category	Distribution[a]				
	North	**West**	**Central**	**East**	**South**
Protozoa					
Leishmaniasis					
Cutaneous	X	X	X	X	X
Visceral	X	X	X	X	X
Malaria	X	X	X	X	X
Trypanosomiasis					
Gambian (west)	O	X	X	O	O
Rhodesian (east)	O	O	O	X	X
Trematodes					
Schistosomiasis					
Schistosoma haematobium	X	X	X	X	X
Schistosoma mansoni	X	X	X	X	X
Paragonimiasis	X	X	X	O	O
Cestodes					
Hydatid disease	X	X	O	X	O
Nematodes					
Filariasis					
Wuchereria bancrofti	X	X	X	X	O
Loa loa	O	X	X	X	O
Onchocerca volvulus	O	X	X	X	O

[a] X, present; O, absent.

TABLE A1.5 Distribution of selected parasitic infections in Asia

Category	Distribution[a]			
	Southwest	**Central South**	**Southeast**	**East**
Protozoa				
Leishmaniasis				
Cutaneous	X	X	O	O
Visceral	O	X	O	O
Malaria	X	X	X	X
Trematodes				
Fasciolopsiasis	O	X	X	X
Clonorchiasis, opisthorchiasis	O	X	X	X
Paragonimiasis	O	X	X	X
Schistosomiasis				
Schistosoma haematobium	X	India	O	O
S. japonicum	O	O	X	X
S. mansoni	O	O	O	O
Cestodes				
Diphyllobothriasis	O	O	O	X
Hydatid disease	X	O	X	O
Nematodes				
Filariasis				
Dirofilariasis	X	X	X	Japan, Korea
Wuchereria bancrofti	O	X	X	X
Brugia malayi	O	India	X	X

[a] X, present; O, absent.

TABLE A1.6 Distribution of selected parasitic infections in Oceania

Category	Distribution[a]					
	Australia	New Guinea	South Pacific	Polynesia	West Pacific	Hawaii
Protozoa						
Malaria	O	X	X	O	X	O
Trematodes						
Paragonimiasis	O	O	X	X	O	O
Nematodes						
Filariasis						
Dirofilariasis	X	X	X	X	X	N
Wuchereria bancrofti	X	X	X	X	X	O
Eosinophilic meningitis	X	O	X	X	X	X

[a] X, present; O, absent; N, no data.

TABLE A1.7 Cosmopolitan distribution of common parasitic infections (North America, Mexico, Central America, South America, Europe, Africa, Asia, and Oceania)

Category	Species
Protozoa	
Intestinal	*Blastocystis* spp.
	Cryptosporidium spp.
	Cyclospora cayetanensis
	Dientamoeba fragilis
	Entamoeba histolytica
	Entamoeba dispar[a]
	Giardia lamblia
	Cystoisospora belli
	Microsporidia[b]
Tissue	*Toxoplasma gondii*
	Microsporidia[b]
Other	*Balamuthia mandrillaris*
	Microsporidia[b]
	Naegleria and *Acanthamoeba* spp.
	Trichomonas vaginalis
Cestodes	*Hymenolepis nana*
	Taenia saginata
Nematodes	
Intestinal	*Ascaris lumbricoides*
	Enterobius vermicularis
	Hookworm
	Strongyloides stercoralis
	Trichuris trichiura
Tissue	*Trichinella* spp.

[a] The name *Entamoeba histolytica* is used to designate the true pathogen, while the name *E. dispar* is now being used to designate the nonpathogen. However, unless trophozoites containing ingested red blood cells (*E. histolytica*) are seen, the two organisms cannot be differentiated on the basis of morphology.

[b] Microsporidia have now been reclassified with the fungi.

TABLE A1.8 Body sites and specimen collection[a]

Site	Specimen options	Collection method[b]
Blood	Smears of whole blood	Thick and thin films, fresh or with anticoagulant
	Anticoagulated blood	Anticoagulant EDTA (1st choice) Heparin (2nd choice)
Bone marrow	Aspirate	Sterile
Central nervous system	Spinal fluid	Sterile
	Brain biopsy specimen	Sterile
Cutaneous ulcers	Aspirates from below surface	Sterile plus air-dried smears
	Biopsy specimen	Sterile, nonsterile to histopathology (formalin acceptable)
Eyes	Biopsy specimen	Sterile (in saline), nonsterile to histopathology
	Scrapings	Sterile (in saline)
	Contact lens	Sterile (in saline)
	Lens solution	Sterile (unopened commercial solutions not acceptable)
Intestinal tract	Fresh stool	0.5-pt (ca. 0.237-liter) waxed container
	Preserved stool[c]	5 or 10% formalin, MIF, SAF, Schaudinn's fluid (mercury, copper, or zinc base)(with PVA), single-vial systems/Universal Fixative (no mercury, no formalin, no PVA)
	Sigmoidoscopy material	Fresh, PVA, or Schaudinn's smears
	Duodenal contents	Entero-Test or aspirates
	Anal impression smear	Cellulose tape (pinworm examination) or other collection device
	Adult worm or worm segments	Saline, 70% alcohol
Liver, spleen	Aspirates	Sterile, collected in 4 separate aliquots (liver)
	Biopsy specimen	Sterile, nonsterile to histopathology
Lungs	Sputum	True sputum (not saliva)
	Induced sputum	Sterile, air-dried smears
	Bronchoalveolar lavage fluid	Sterile, air-dried smears
	Transbronchial aspirate	Sterile, air-dried smears
	Tracheobronchial aspirate	Sterile, air-dried smears
	Brush biopsy specimen	Sterile, air-dried smears
	Open-lung biopsy specimen	Sterile
	Aspirate	Sterile, air-dried smears
Muscle	Biopsy specimen	Fresh, squash preparation, nonsterile to histopathology (formalin acceptable)
Skin	Scrapings	Aseptic, smear or vial
	Skin snip	No preservative
	Biopsy specimen	Sterile (in saline), nonsterile to histopathology
Urogenital system	Vaginal discharge	Saline swab, transport swab (no charcoal), culture medium, plastic-envelope culture; air-dried smear for FA
	Urethral discharge	Same as above
	Prostatic secretions	Same as above
	Urine	Single unpreserved specimen (early morning), 24-h unpreserved specimen

[a] Modified from Garcia LS, ed, *Clinical Microbiology Procedures Handbook*, 3rd ed, ASM Press, Washington, DC, 2010.

[b] FA, fluorescent antibody; MIF, merthiolate-iodine-formalin; PVA, polyvinyl alcohol; SAF, sodium acetate-acetic acid-formalin.

[c] A number of new stool fixatives are now available; some use a zinc sulfate base rather than mercuric chloride. Some collection vials can be used as a single-vial system; both the concentration and permanent stained smear can be performed from the preserved stool. However, not all single-vial systems provide material that can be used for fecal immunoassay procedures. The Universal Fixative is now available and can be used for the ova and parasite examination, most fecal immunoassays, and some PCR (fixative has no formalin, no mercury, and no PVA).

TABLE A1.9 Body sites and possible parasites recovered (trophozoites, cysts, oocysts, spores, adults, larvae, eggs, amastigotes, and trypomastigotes)[a]

Site	Parasites
Blood	
Red cells	*Plasmodium* spp.
	Babesia spp.
Leukocytes	*Leishmania* spp.
	Toxoplasma gondii
Whole blood or plasma	*Trypanosoma* spp.
	Microfilariae
Bone marrow	*Leishmania* spp.
	Trypanosoma cruzi
	Plasmodium spp.
Central nervous system	*Taenia solium* (cysticerci)
	Echinococcus spp.
	Naegleria fowleri
	Acanthamoeba and *Hartmanella* spp.
	Balamuthia mandrillaris
	Sappinia diploidea
	Toxoplasma gondii
	Microsporidia[b]
	Trypanosoma spp.
Cutaneous ulcers	*Leishmania* spp.
	Acanthamoeba spp.
	Entamoeba histolytica
Eyes	*Acanthamoeba* spp.
	Toxoplasma gondii
	Loa loa
	Microsporidia[b]
	Dirofilaria spp.
	Thelazia spp.
Intestinal tract	*Entamoeba histolytica*
	Entamoeba dispar, *Entamoeba moshkovskii*, *Entamoeba bangladeshi*
	Entamoeba coli
	Entamoeba hartmanni
	Endolimax nana
	Iodamoeba bütschlii
	Blastocystis spp.
	Giardia lamblia
	Chilomastix mesnili
	Dientamoeba fragilis
	Pentatrichomonas hominis
	Balantidium coli
	Cryptosporidium spp.
	Cyclospora cayetanensis
	Cystoisospora belli
	Encephalitozoon spp.
	Enterocytozoon bieneusi
Intestinal tract *(cont.)*	Microsporidia[b]
	Ascaris lumbricoides
	Enterobius vermicularis
	Hookworm
	Strongyloides stercoralis
	Trichuris trichiura
	Hymenolepis nana
	Hymenolepis diminuta
	Taenia saginata
	Taenia solium
	Diphyllobothrium latum
	Clonorchis (*Opisthorchis*) *sinensis*
	Paragonimus spp.
	Schistosoma spp.
	Fasciolopsis buski
	Fasciola hepatica
	Metagonimus yokogawai
	Heterophyes heterophyes
Liver and spleen	*Echinococcus* spp.
	Entamoeba histolytica
	Leishmania spp.
	Microsporidia[b]
	Capillaria hepatica
	Clonorchis (*Opisthorchis*) *sinensis*
Lungs	*Pneumocystis jiroveci*[b]
	Cryptosporidium spp.[c]
	Echinococcus spp.
	Paragonimus spp.
	Toxoplasma gondii
	Helminth larvae
Muscles	*Taenia solium* (cysticerci)
	Trichinella spp.
	Onchocerca volvulus (nodules)
	Trypanosoma cruzi
	Microsporidia[b]
Skin	*Entamoeba histolytica*
	Acanthamoeba spp.
	Balamuthia mandrillaris
	Leishmania spp.
	Onchocerca volvulus
	Microfilariae
Urogenital system	*Trichomonas vaginalis*
	Schistosoma spp.
	Microsporidia[b]
	Microfilariae

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[a] Modified from Garcia LS, ed, *Clinical Microbiology Procedures Handbook*, 3rd ed, ASM Press, Washington, DC, 2010. This table does not include every possible parasite that could be found in a particular body site. However, the most likely organisms have been listed.

[b] Now classified with the fungi.

[c] Disseminated in severely immunosuppressed individuals.

TABLE A1.10 Body site, specimen and procedures, recommended methods, relevant parasites, and comments[a]

Body site	Procedures and specimens	Recommended methods and relevant parasites[b]	Comments
Amniotic fluid	PCR (and/or culture): fresh material	PCR based on the detection of highly repetitive gene sequences is the method of choice.	Only applicable to confirm suspected prenatal *Toxoplasma* infections.
	Animal inoculation (toxoplasmosis)		
Blood	Microscopy[b]: thin and thick blood films; fresh blood or EDTA blood (fill EDTA tube completely with blood, then mix)	Giemsa or other blood stains (all blood parasites); hematoxylin-based stain (sheathed microfilariae); for malaria, thick and thin blood films are mandatory and should be prepared within 30–60 min of blood collection via venipuncture (other tests may be used as well); Wright-Giemsa stain or Diff-Quik (rapid stains) can also be used.	Most drawings and descriptions of blood parasites are based on Giemsa-stained blood films. Although Wright's stain (or Wright-Giemsa combination stain) will work, stippling in malaria may not be visible and the organisms' colors will not match the descriptions. However, with other stains (those listed above, in addition to some of the "rapid" blood stains), the organisms are detectable on the blood films. The use of blood collected with anticoagulant (rather than fresh) has direct relevance to the morphology of malaria organisms seen in peripheral blood films. If the blood smears are prepared after more than 1 h, stippling may not be visible, even if the correct pH buffers are used. Also, if blood is kept at room temperature (with the stopper removed), the male microgametocyte may exflagellate and fertilize the female macrogametocyte, and development continues within the tube of blood as it would in the mosquito host. The ookinete may actually resemble a *Plasmodium falciparum* gametocyte. The microgamete may resemble spirochetes: white blood cells serve as the internal quality control for every blood film; if the leukocytes exhibit acceptable morphology, parasites present will also be acceptable per staining and morphology characteristics.
	Concentration methods: EDTA blood	Buffy coat, fresh blood films for detection of moving microfilariae or trypanosomes; QBC, a screening method for blood parasites (hematocrit tube contains acridine orange), has been used for malaria, *Babesia*, trypanosomes, and microfilariae; it is usually impossible to identify malaria organisms to the species level; this requires high levels of training; confirmation to species often requires examination of thin blood films.	
	Antigen detection: EDTA-blood for malaria, serum or plasma for circulating antigens (hemolyzed blood can interact in some tests)	Commercial test kits for malaria and some microfilariae	
	PCR: EDTA-blood, ethanol-fixed or unfixed thin and thick blood films, coagulated blood, possible with hemolyzed or frozen blood samples	Sensitivity not higher than thick films for *Plasmodium* spp.; much more sensitive for *Leishmania* (peripheral blood is used from immunodeficient patients only). Sequencing of PCR product is often used for species or genotype identification.	So far no commercial tests are available; some reference laboratories offer PCR or other molecular procedures for all five species of human malaria, *Babesia*, *Leishmania*, and trypanosomes. High laboratory standards required (may work with frozen, coagulated, or hemolyzed blood samples).
	Specific antibody detection: serum or plasma, anticoagulated or coagulated blood (hemolyzed blood can cause problems in some tests)	Most commonly used are EIA (many test kits commercially available), EITB (commercially available for some parasites), and IFA.	Many laboratories are using in-house tests; only a few fully defined antigens are available; sensitivities and specificities of the tests should be validated by the laboratory.
Bone marrow	Biopsy specimens or aspirates Microscopy: thin and thick films with aspirate collected in EDTA	Giemsa or other blood stains (including rapid stains) (all blood parasites)	*Leishmania* amastigotes are recovered in cells of the reticuloendothelial system; if films are not prepared directly after sample collection, infected cells may disintegrate. Sensitivity of microscopy is low, so it should be used only in combination with other methods.

	Cultures: sterile material in EDTA or culture medium	Culture for *Leishmania* (or *Trypanosoma cruzi*)	Positive control cultures should be performed with patient cultures.
	PCR: aspirate in EDTA	PCR for blood parasites including *Leishmania* and *Toxoplasma* and rare other parasites	
Central nervous system	Microscopy: spinal fluid and CSF (wet examination, stained smears), brain biopsy specimen (touch or squash preparations, stained)	Stains: Giemsa or other blood stains (trypanosomes, *Toxoplasma*); Giemsa, trichrome, or calcofluor (amebae [*Naegleria* or *Sappinia*—PAM; *Acanthamoeba* or *Balamuthia*—GAE]); Giemsa, acid-fast, PAS, modified trichrome, silver methenamine (microsporidia) (tissue Gram stains also recommended for microsporidia in routine histologic preparations); H&E, routine histology (larval cestodes, *Taenia solium* cysticerci, *Echinococcus* spp.)	If CSF is received (with no suspect organism suggested), Giemsa would be the best choice; however, modified trichrome or calcofluor is also recommended as a second stain (amebic cysts, microsporidia). If brain biopsy material is received (particularly from an immunocompromised patient), cultivation is recommended for microsporidial isolation and PCR for identification to the species or genotype level. A small amount of the sample should always be stored frozen for PCR analyses in case the results of the other methods are inconclusive.
	Culture: sterile aspirate or biopsy material (in physiologic NaCl)	Free-living amebae (exception: *Balamuthia* does not grow in the routine agar/bacterial overlay method), microsporidia, and *Toxoplasma* (can be cultured in tissue culture media)	
	PCR: aspirate or biopsy material fresh, frozen, or fixed in ethanol	Protozoa and helminths, species and genotype characterization	
Cutaneous ulcers	Microscopy: aspirate, biopsy (smears, touch or squash preparations, histologic sections) Cultures (less common) PCR: aspirate, biopsy material fresh, frozen, or fixed in ethanol	Giemsa or other blood stains (*Leishmania*); H&E, routine histology (*Acanthamoeba* spp., *Entamoeba histolytica*) *Leishmania* (often bacterial contamination, culture problems), free-living amebae, *Leishmania* (species identification)	The most likely causative parasites would be *Leishmania*, which would stain with Giemsa. PAS could be used to differentiate *Histoplasma capsulatum* from *Leishmania* in tissue. In immunocompromised patients, skin ulcers have been documented with *Acanthamoeba* or *Entamoeba histolytica* amebae as causative agents; cultures of material from cutaneous ulcers may be contaminated with bacteria; PCR would be the method of choice.
Eyes	Microscopy: smears, touch or squash preparations; biopsy specimens, scrapings, contact lens, sediment of lens solution	Calcofluor for cyst only (amebae [*Acanthamoeba*]); Giemsa for trophozoites and cysts (amebae); modified trichrome (preferred) or silver methenamine stain, PAS, acid-fast (microsporidial spores), H&E, routine histology (cysticerci, *Loa loa*, *Toxoplasma*)	Some free-living amebae (most commonly *Acanthamoeba*) have been implicated as a cause of keratitis. Although calcofluor stains the cyst walls, it does not stain the trophozoites. Therefore, in suspect cases of amebic keratitis, both stains should be used. H&E (routine histology) can be used to detect and confirm cysticercosis. The adult worm of *Loa loa*, when removed from the eye, can be stained with a hematoxylin-based stain (Delafield's) or can be stained and examined by routine histology. Microsporidia: confirmation to the species or genotype levels can be done by PCR and sequence analyses or EM; however, the spores could be found by routine light microscopy with modified trichrome, calcofluor, and/or tissue Gram stains.
	Culture: fresh material (see above) in PBS supplemented with antibiotics if possible to avoid bacterial growth	Cultures: free-living amebae, *Toxoplasma*, microsporidia	
	PCR: fresh material in physiologic NaCl or PBS, ethanol or frozen	Free-living amebae, *Toxoplasma*, microsporidian species and genotype identification	

(continued)

TABLE A1.10 Body site, specimen and procedures, recommended methods, relevant parasites, and comments[a] *(continued)*

Body site	Procedures and specimens	Recommended methods and relevant parasites[b]	Comments
Intestine	Stool and other intestinal material		Stool fixation with formalin or formalin-containing fixatives preserves parasite morphology (trophozoites marginal), allows prolonged storage (room temperature) and long transportation, and prevents hatching of *Schistosoma* eggs, but makes *Strongyloides* larval concentration difficult and impedes further PCR analyses. Single-vial Universal Fixative is now available (no mercury, no formalin, no PVA); can be used for O&P, most fecal immunoassays, special stains, some PCR
	Microscopy: stool, sigmoidoscopy material, duodenal contents (all fresh or preserved), direct wet smear, concentration methods	Concentration methods: ethyl acetate sedimentation of SAF-fixed stool samples (most protozoa); flotation or combined sedimentation flotation methods (helminth ova); agar or Baermann concentration (larvae of *Strongyloides* spp., fresh stool required)	Agar plate for *Strongyloides* is more sensitive than routine O&P.
		Direct wet smear (direct examination of unpreserved fresh material is also used (motile protozoan trophozoites; helminth eggs, protozoan cysts may also be detected).	Taeniid eggs cannot be identified to the species level.
		Stains: trichrome or iron hematoxylin (intestinal protozoa); modified trichrome (microsporidia); modified acid-fast (*Cryptosporidium*, *Cyclospora*, *Cystoisospora*)	Microsporidia: confirmation to the species or genotype levels requires PCR or EM; however, modified trichrome and/or calcofluor stains can be used to confirm the presence of spores.
	Anal impression smear	Adhesive cellulose tape, no stain (*Enterobius vermicularis*)	Four to six consecutive negative tapes are required to rule out infection with pinworm (*Enterobius vermicularis*).
	Adult worms or tapeworm segments (proglottids)	Carmine stains (rarely used for adult worms or cestode segments). Proglottids can usually be identified to the genus level (*Taenia*, *Diphyllobothrium*, *Hymenolepis*) without using tissue stains.	Worm segments can be stained with special stains. However, after dehydration through alcohols and xylenes (or xylene substitutes) without prior staining, the sexual organs and the branched uterine structure will be visible, allowing identification of the proglottid to the species level, providing the proglottids are gravid.
	Antigen detection (fresh or frozen material, suitability of fixation is test dependent)	Commercial immunoassays, e.g., EIA, FA, cartridge formats (*Entamoeba histolytica*, the *Entamoeba histolytica/E. dispar* group, *Giardia lamblia*, *Cryptosporidium* spp.); in-house tests for *T. solium* and *T. saginata* and other parasites	Coproantigens can be detected in the prepatent period and independently from egg excretion.
	PCR: fresh, frozen, or ethanol-fixed material	Few commercial tests available; primers for genus or species identification of most helminths and protozoa are published; commercial instrumentation available, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium*, *Cyclospora cayetanensis*	Due to potential inhibition after DNA extraction from stool samples, concentration or isolation methods may be required prior to DNA extraction. However, new DNA isolation kits facilitate isolation of high-quality DNA from stool. Sequence analyses may be required for species or genotype identification.

	Biopsy specimens		
	Microscopy: fixed for histology or touch or squash preparations for staining	H&E, routine histology (*Entamoeba histolytica*, *Cryptosporidium*, *Cyclospora*, *Cystoisospora belli*, *Giardia*, microsporidia); less common findings would include *Schistosoma* spp., hookworm, or *Trichuris*	Special stains may be helpful for the identification of microsporidia: tissue Gram stains, silver stains, PAS, and Giemsa or modified acid-fast stains for the coccidia
Liver and spleen	Biopsy specimens or aspirates Microscopy: unfixed material in physiologic NaCl; fixed for histology	Examination of wet smears for *Entamoeba histolytica* (trophozoites), protoscolices of *Echinococcus* spp. or eggs of *Capillaria hepatica*; Giemsa (*Leishmania*, other protozoa and microsporidia); H&E (routine histology)	There are definite risks associated with punctures (aspirates and/or biopsy) of spleen or liver lesions (*Echinococcus*). Always keep a small amount of material frozen for PCR.
	Culture: sterile preparation of fresh material	For *Leishmania* (not common)	
	Animal inoculation: sterile preparation of fresh material	Intraperitoneal inoculation of *E. multilocularis* cyst material for viability test after long-term chemotherapy	
	PCR: fresh, frozen, or ethanol fixed	Species or genotype identification (e.g., *Echinococcus* spp.)	
Respiratory tract	Sputum, induced sputum, nasal and sinus discharge, bronchoalveolar lavage fluid, transbronchial aspirate, tracheobronchial aspirate, brush biopsy specimen, open-lung biopsy specimen Microscopy: unfixed material, treated for smear preparation	Helminth larvae (*Ascaris*, *Strongyloides*), eggs (*Paragonimus*, *Capillaria*), or hooklets (*Echinococcus*) can be recovered in unstained respiratory specimens; stains include Giemsa for many protozoa including *Toxoplasma* tachyzoites, modified acid-fast stains (*Cryptosporidium*), and modified trichrome (microsporidia); routine histology with H&E; silver methenamine stain, PAS, acid-fast, and tissue Gram stains for helminths, protozoa, and microsporidia	Immunoassay reagents (FA) are available for the diagnosis of pulmonary cryptosporidiosis (may require validation). Routine histologic procedures allow the identification of any of the helminths or helminth eggs present in the lungs. Disseminated toxoplasmosis and microsporidiosis are well documented, with organisms being found in many different respiratory specimens.
	PCR: fresh, frozen, or fixed in ethanol		
Muscles	Biopsy material Microscopy: unfixed, touch and squash preparations or fixed for histology and EM	Larvae of *Trichinella* spp. can be identified unstained (species identification with single larvae by PCR); H&E, routine histology (*Trichinella* spp., cysticerci); silver methenamine stain, PAS, acid-fast, tissue Gram stains, EM (several genera of microsporidia)	If *Trypanosoma cruzi* is present in the striated muscle, the organisms could be identified in routine histology preparations. Modified trichrome, tissue Gram stains, and/or calcofluor stains can be used to confirm the presence of microsporidial spores.
	PCR: fresh, frozen, or ethanol fixed	Microsporidian identification to the species level requires subsequent sequencing.	
Skin	Aspirates, skin snips, scrapings, biopsy specimens	See cutaneous ulcer (above).	Any of the potential parasites present can be identified by routine histology procedures.

(continued)

TABLE A1.10 Body site, specimen and procedures, recommended methods, relevant parasites, and comments[a] *(continued)*

Body site	Procedures and specimens	Recommended methods and relevant parasites[b]	Comments
	Microscopy: wet examination, stained smear (or fixed for histology or EM)	Wet preparations (microfilariae), Giemsa-stained smears or H&E, routine histology (*Onchocerca volvulus*, *Dipetalonema streptocerca*, *Dirofilaria repens*, other larvae causing cutaneous larva migrans [zoonotic *Strongyloides* spp.], hookworms), *Leishmania*, *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Entamoeba histolytica*, microsporidia, and arthropods (*Sarcoptes* and other mites)	
	PCR: fresh, frozen, or fixed in ethanol	Primers for most relevant parasite species available	
Urogenital system	Vaginal discharge, saline swab, transport swab (no charcoal), air-dried smear for FA, urethral discharge, prostatic secretions, urine (single unpreserved, 24-h unpreserved, or early-morning specimens)	Giemsa, immunoassay reagents (FA) (*Trichomonas vaginalis*); Delafield's hematoxylin (microfilariae); modified trichrome (microsporidia); H&E, routine histology PAS, acid-fast, tissue Gram stains (microsporidia); direct examination of urine sediment for *Schistosoma haematobium* eggs or microfilariae	Although *T. vaginalis* is probably the most common parasite identified, there are others to consider, the most recently implicated organisms being in the microsporidian group. Microfilariae could also be recovered and stained. Fixation of urine with formalin prevents hatching of *Schistosoma* eggs. Perform concentration using saline to prevent premature hatching of schistosome eggs. Material must be put into culture medium immediately after collection; do not cool or freeze (*T. vaginalis*).
	Microscopy: wet smears, smears of urine sediment, stained smears	Identification and propagation of *T. vaginalis* (commercialized plastic-envelope culture systems available); moving trophozoites can be detected using microscopy (or in Giemsa-stained smears)	
	Cultivation: vaginal or urethral discharge or swab preparations		
	Antigen detection (vaginal swab)	Commercial immunoassay (rapid formal), *T. vaginalis*	
	Probe	Commercial instrumentation, *T. vaginalis*	
	PCR: fresh, frozen, or fixed in ethanol		

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[a] CSF, cerebrospinal fluid; EIA, enzyme immunoassay; EITB, enzyme-linked immunoelectrotransfer blot (Western blot); EM, electron microscopy; FA, fluorescent antibody; GAE, granulomatous amebic encephalitis; GI, gastrointestinal; H&E, hematoxylin and eosin; IFA, indirect fluorescent-antibody assay; O&P, ova and parasite exam; PAM, primary amebic meningoencephalitis; PAS, periodic acid-Schiff stain; PBS, phosphate-buffered saline; QBC, quantitative buffy coat; SAF, sodium acetate-acetic acid-formalin.

[b] Many parasites or parasite stages may be detected in standard histologic sections of tissue material. However, species identification is difficult and additional examinations may be required. Usually, these techniques are not considered first-line methods. Additional methods like EM (electron microscopy) are carried out only by specialized laboratories and are not available for standard diagnostic purposes. EM examination for species identification has largely been replaced by PCR assays.

TABLE A1.11 Examination of tissue and body fluids[a]

Suspected causative agent	Disease(s)	Appropriate test(s)[b]	Positive result
Protozoa			
Naegleria fowleri	Primary amebic meningoencephalitis (PAM)	1. Wet examination of CSF (not in counting chamber) 2. Stained preparation of CSF sediment	Trophozoites present and identified
Acanthamoeba spp.	Amebic keratitis, chronic meningoencephalitis (granulomatous amebic encephalitis [GAE]); skin lesions	1. Culture or stained smears 2. Calcofluor (cysts only) 3. Biopsy for routine histology	Trophozoites and/or cysts present and identified
Balamuthia mandrillaris	Chronic meningoencephalitis (GAE)	1. Calcofluor (cysts only) 2. Biopsy for routine histology	Trophozoites and/or cysts present and identified
Entamoeba histolytica	Amebiasis; cutaneous, intestinal, extraintestinal	1. Biopsy for routine histology 2. Immunoassays	Trophozoites present and identified
Giardia lamblia	Giardiasis, intestinal	1. Duodenal aspirate 2. Duodenal biopsy or routine histology 3. Entero-Test capsule 4. Immunoassays	Trophozoites and/or cysts present and identified
Leishmania spp.			
Cutaneous lesions	Cutaneous leishmaniasis	1. Material from under bed of ulcer A. Smear B. Culture C. Animal inoculation 2. Punch biopsy A. Routine histology B. Squash preparation C. Culture D. Animal inoculation	Amastigotes recovered in macrophages of skin or from animal inoculation; other stages recovered in culture
Mucocutaneous lesions	Mucocutaneous leishmaniasis	As for cutaneous leishmaniasis	Amastigotes recovered in macrophages of skin and mucous membranes or from inoculation of animals; other stages recovered in culture
Visceral	Visceral leishmaniasis (kala azar)	1. Buffy coat A. Stain B. Culture C. Animal inoculation 2. Bone marrow A. Stain B. Culture C. Animal inoculation 3. Liver or spleen biopsy A. Routine histology B. Stain C. Culture D. Animal inoculation	Amastigotes recovered in cells of reticuloendothelial system; other stages recovered in culture

(continued)

TABLE A1.11 Examination of tissue and body fluids[a] *(continued)*

Suspected causative agent	Disease(s)	Appropriate test(s)[b]	Positive result
Toxoplasma gondii	Toxoplasmosis	1. Lymph node biopsy A. Routine histology B. Tissue culture isolation C. Animal inoculation 2. Serology	Identification of organisms plus appropriate serologic test results
Cryptosporidium spp.	Cryptosporidiosis	1. Duodenal scraping 2. Duodenal biopsy A. Stain B. Routine histology 3. Other tissue biopsy A. Routine histology B. Squash preparation 4. Sputum (modified AFB stains) 5. Immunoassays	Identification of organisms in microvillus border or in other tissues (lung and gallbladder have also been involved)
Microsporidia[c] *Nosema* spp. *Encephalitozoon* spp. *Enterocytozoon* spp. *Pleistophora* spp. *Trachipleistophora* spp. *Anncaliia* spp. *Microsporidium* spp. *Vittaforma corneae* *Tubulinosema* sp.	Microsporidiosis (now classified with the fungi)	Routine histology (fair); acid-fast, PAS, modified trichrome, Giemsa, and tissue Gram stains recommended (spores); animal inoculation not recommended (latent infections); EM or PCR may be necessary to confirm genus	These organisms (spores) have been found as insect or other animal parasites; the most common route of infection is probably ingestion. Human cases involve gastrointestinal tract, muscle, and CSF (AIDS); other body sites have also been documented; not limited to compromised patients
Helminths			
Larvae (*Ascaris lumbricoides*, *Strongyloides stercoralis*)	"Pneumonia"	Sputum, wet preparation	This is an incidental finding but has been reported in severe infections
Eggs (*Paragonimus* spp.)	Paragonimiasis	Sputum, wet preparation Stool, routine O&P	Eggs will be coughed up and will appear as "iron filings"; eggs can also be found in stool
Hooklets (*Echinococcus* spp.)	Hydatid disease	Sputum, wet preparation	Rare finding, but hooklets can be found when the hydatid cyst is in the lungs
Onchocerca volvulus, *Mansonella streptocerca*	Onchocerciasis	Skin	Skin snips examined in saline; microfilariae may be present
Schistosoma spp.	Schistosomiasis	1. Rectal valve biopsy 2. Bladder biopsy	Eggs present and identified

[a] Modified from Garcia LS, ed, *Clinical Microbiology Procedures Handbook*, 3rd ed, ASM Press, Washington, DC, 2010. AFB, acid-fast bacillus; CSF, cerebrospinal fluid; EM, electron microscopy; O&P, ova and parasite exam; PAS, periodic acid-Schiff.

[b] PCR is now being used for the identification of a number of human parasites; however, these tests are generally limited to academic or reference centers. Routine laboratory use is not common.

[c] Now classified with the fungi.

TABLE A1.12 Key characteristics of protozoa of the intestinal tract and urogenital system[a]

Organism	Trophozoite or tissue stage	Cyst or other stage in specimen	Comments
Amebae			
Entamoeba histolytica (pathogenic)	Cytoplasm is clean; presence of RBCs is diagnostic, but cytoplasm may also contain some ingested bacteria; peripheral nuclear chromatin is evenly distributed with central, compact karyosome.	Mature cyst contains 4 nuclei; chromatoidal bars have smooth, rounded ends; cannot be differentiated from *E. dispar*	Considered pathogenic; should be reported to Public Health; trophozoites can be confused with macrophages and cysts can be confused with PMNs in the stool.
Entamoeba dispar (nonpathogenic)	Morphology identical to that of *E. histolytica* (confirmed by presence of RBCs in cytoplasm). If no RBCs present, molecular testing or fecal immunoassays are necessary to confirm species designation.	Mature cyst has morphology identical to that of *E. histolytica*.	Nonpathogenic; morphology resembles *E. histolytica*; these organisms should be reported as *Entamoeba histolytica/E. dispar* and reported to Public Health; immunoassay reagents are now available to identify the *E. histolytica/E. dispar* group and to differentiate pathogenic *E. histolytica* and nonpathogenic *E. dispar*; some laboratories may decide to use these reagents on a routine basis, depending on positivity rate and cost.
Entamoeba histolytica/E. dispar (some like to add the word "group" to indicate the two organisms cannot be differentiated on the basis of morphology unless RBCs are seen within the cytoplasm or *E. histolytica* is confirmed using species-specific immunoassays.			Correct way to report, unless species-specific immunoassay is used to identify *E. histolytica* or trophozoites are seen with ingested RBCs (*E. histolytica*). *E. moshkovskii* and *E. bangladeshi* look very similar to the *E. histolytica/E. dispar* group. Some have recommended using the term "*Entamoeba* complex."
Entamoeba hartmanni (nonpathogenic)	Looks identical to *E. histolytica/E. dispar* but is smaller (<12 µm); RBCs are not ingested.	Mature cyst contains 4 nuclei but often has only 2; chromatoidal bars are often present and look like those of *E. histolytica/E. dispar* (size, <10 µm).	Shrinkage occurs on the permanent stain due to dehydration steps (especially in the cyst form). *E. histolytica/E. dispar* may actually be below the 12- and 10-µm cutoff limits and can be as much as 1.5 µm below the limits quoted for wet preparation measurements.
Entamoeba coli (nonpathogenic)	Cytoplasm is dirty and may contain ingested bacteria or debris; peripheral nuclear chromatin is unevenly distributed, with a large, eccentric karyosome.	Mature cyst contains 8 nuclei; more may be seen; chromatoidal bars (if present) tend to have sharp, pointed ends.	If a smear is too thick or thin and if stain is too dark or light, *E. histolytica/E. dispar* and *E. coli* can often be confused, since there is much overlap in morphology.
Endolimax nana (nonpathogenic)	Cytoplasm is clean, not diagnostic, with a great deal of nuclear variation; there may even be some peripheral nuclear chromatin. Normally only karyosomes are visible.	Cyst is round to oval, with the 4 nuclear karyosomes being visible.	There is more nuclear variation in this ameba than any others. The organisms can be confused with *Dientamoeba fragilis* and/or *E. hartmanni*.

(continued)

TABLE A1.12 Key characteristics of protozoa of the intestinal tract and urogenital system[a] *(continued)*

Organism	Trophozoite or tissue stage	Cyst or other stage in specimen	Comments
Iodamoeba bütschlii (nonpathogenic)	Cytoplasm contains much debris; organisms are usually larger than *E. nana* but may look similar; large karyosome.	Cyst contains single nucleus (may be "basket nucleus") with bits of nuclear chromatin arranged on the nuclear membrane (the karyosome is the basket, bits of chromatin are the handle); large glycogen vacuole	Glycogen vacuole stains brown with the addition of iodine in the wet preparation; "basket nucleus" is more common in cyst but can be seen in trophozoite; vacuole may be so large that the cyst collapses on itself.
Blastocystis spp. (pathogenic) undergoing review for possible reclassification; multiple strains or species that look the same (~half are pathogenic, half are nonpathogenic)	Trophozoites may/may not be seen; often in patients with diarrhea; difficult to identify	Central body forms most common; tremendous size variation; central area may or may not stain; outer perimeter contains multiple nuclei (often seen as various sized dots)	These are the most common GI tract protozoa worldwide, much more common than *Giardia* or *Dientamoeba* (tend to be equal or *Dientamoeba* more common than *Giardia* in many areas); symptomatic patients tend to be treated.
Flagellates			
Giardia lamblia (pathogenic)	Trophozoites are teardrop shaped from the front and like a curved spoon from the side; contain nuclei, linear axonemes, and curved median bodies.	Cysts are round to oval, containing multiple nuclei, axonemes, and median bodies.	Organisms live in the duodenum, and multiple stool specimens may be negative; additional sampling techniques (aspiration, Entero-Test) may be needed; fecal immunoassays are helpful.
Chilomastix mesnili (nonpathogenic)	Trophozoites are teardrop shaped; cytostome must be visible for identification.	Cyst is lemon shaped with 1 nucleus and a curved fibril called the shepherd's crook.	The cyst can be identified much more easily than the trophozoite form. The trophozoite looks like some of the other small flagellates.
Dientamoeba fragilis (pathogenic)	Cytoplasm contains debris; may contain 1 or 2 nuclei (chromatin often fragmented into 4 dots).	Cyst form has now been identified; appears to have double wall; percentage is quite low (~1–2%); thus it can be very difficult to find and identify.	Tremendous size and shape range on a single smear; trophozoites with 1 nucleus can resemble *E. nana*.
Trichomonas vaginalis (pathogenic)	Supporting rod (axostyle) is present; undulating membrane comes halfway down the organism; small dots may be seen in the cytoplasm along the axostyle.	No known cyst form	Recovered from genitourinary system; often diagnosed at bedside with wet preparation (motility)
Pentatrichomonas hominis (nonpathogenic)	Supporting rod (axostyle) is present; undulating membrane comes all the way down the organism; small dots may be seen in the cytoplasm along the axostyle.	No known cyst form	Recovered in stool; trophozoites may resemble other small flagellate trophozoites.
Ciliates			
Balantidium coli (pathogenic)	Very large trophozoites (50–100 μm long) covered with cilia; large bean-shaped macronucleus present; very small micronucleus is difficult to see.	Morphology not significant with exception of large, bean-shaped macronucleus; small micronucleus is difficult to see.	Rarely seen in the United States; causes severe diarrhea with large fluid loss; will be seen in proficiency-testing specimens or possibly people who work around pigs

Coccidia			
Cryptosporidium spp. (pathogenic)	Seen in intestinal mucosa (edge of brush border), gallbladder, and lungs; present in biopsy specimens	Oocysts seen in stool and/or sputum; organisms acid fast, measure 4–6 µm; hard to find if only a few are present	Chronic infection in the compromised host (internal autoinfective cycle), self-cure in the immunocompetent host; numbers of oocysts correlate with stool consistency; can cause severe, watery diarrhea; oocysts are immediately infective when passed.
Cyclospora cayetanensis (pathogenic)	Experience with this organism is not extensive; may be difficult to identify in tissue; since patients are immunocompetent, biopsy specimens will rarely be required or requested.	Oocysts seen in stool; approximately 8–10 µm in size; are unsporulated and thus difficult to recognize as coccidia; mimic *Cryptosporidium* on modified acid-fast-stained smears	Most infections are associated with the immunocompetent individual but may also be seen in the immunosuppressed patient; may be associated with traveler's diarrhea; oocysts are not immediately infective when passed. Within the United States, infections have been associated with contaminated food, including raspberries, basil, snow peas, and mesclun (baby lettuce leaves). PCR can detect 40 or fewer oocysts per 100 g of raspberries or basil but has a detection limit of around 1,000 per 100 g in mesclun lettuce.
Cystoisospora belli (pathogenic)	Seen in intestinal mucosal cells; seen in biopsy specimens; not as common as *Cryptosporidium*	Oocysts seen in stool; organisms are acid fast; best technique is concentration, not permanent stained smear	Thought to be the only *Cystoisospora* sp. that infects humans; oocysts are not immediately infective when passed.
Microsporidia[b] *Nosema* spp. *Encephalitozoon* spp. *Pleistophora* spp. *Trachipleistophora* spp. *Anncaliia* spp. *Enterocytozoon* spp. *Microsporidium* spp. *Vittaforma corneae* *Tubulinosema* sp.	Developing stages sometimes difficult to identify; spores can be identified by size, shape, and presence of polar tubules.	Depending on the genus involved, spores could be identified in stool or urine using the modified trichrome stain, calcofluor, or immunoassay reagents (available outside U.S.).	Spores are generally quite small (1–2.0 µm for *Enterocytozoon* spp.) and can easily be confused with other organisms or artifacts (particularly in stool). Infections tend to be present in immunosuppressed patients; however, they are not limited to this patient group.

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[a] Modified from Garcia LS, ed, *Clinical Microbiology Procedures Handbook*, 3rd ed, ASM Press, Washington, DC, 2010. GI, gastrointestinal; PMN, polymorphonuclear neutrophils; RBC, red blood cells.

[b] Now classified with the fungi.

TABLE A1.13 Key characteristics of tissue protozoa[a]

Species	Shape and size	Other features[b]
Toxoplasma gondii		
Trophozoites (tachyzoites)	Crescent shaped; 4–6 µm long by 2–3 µm wide	Found in peritoneal fluid of experimentally infected mice; intracellular forms somewhat smaller and not usually seen in humans. May be isolated in tissue culture, particularly from CSF. Diagnosis is most frequently based on clinical history and serologic evidence (acute- and convalescent-phase sera).
Cysts (bradyzoites)	Generally spherical; 200 µm to 1 mm in diameter	Occur in many tissues (approximately 25% of the U.S. population have these organisms in the tissues, indicating past infection). Many infections are asymptomatic. Infections in the compromised host are very serious and involve the CNS. In these patients, particularly those with AIDS, diagnostic serologic titers may be very difficult to demonstrate and/or interpret. **Note** Organisms identified in histologic preparations or isolated in animals or tissue culture systems may or may not be the causative agent of the symptoms.
Cryptosporidium spp.	Oocyst usually round, 4–6 µm; each mature oocyst contains sporozoites (infective on passage)	Oocyst is usually the diagnostic stage in stool, sputum, and possibly other body specimens. Various other stages in the life cycle can be seen in biopsy specimens taken from the gastrointestinal tract (brush border of epithelial cells and intestinal tract) and other tissues (lung and gallbladder). A number of modified acid-fast stains have been used successfully. Detection methods using immunoassay reagents are also available. **Note** Infection in immunocompetent hosts is self-limiting; however, in immunodeficient patients (AIDS), the infection is chronic owing to an autoinfective capability in the life cycle. The number of oocysts usually correlates with the symptoms (watery diarrhea indicates many oocysts in the specimen). The more normal the stool, the more difficult it is to find the oocysts. Clinicians should be aware that risk groups include animal handlers, travelers, immunocompromised individuals, children in day care centers, and anyone who comes in contact with these individuals. Since the oocysts are immediately infective, nosocomial transmission has been documented.
Cyclospora cayetanensis	Oocyst usually round, 8–10 µm; each oocyst is immature on passage; no internal morphology visible; oocysts appear as "wrinkled cellophane"	The oocyst is the diagnostic stage in stool. Various other stages in the life cycle can be seen in biopsy specimens taken from the gastrointestinal tract (within epithelial cells and intestinal tract); oocysts are not immediately infectious when passed. The morphology is similar to that of *Cystoisospora belli*. A number of modified acid-fast stains have been used successfully to demonstrate the oocysts (quite acid-fast variable). Detection methods involving immunoassay reagents are under development.
Cystoisospora belli	Ellipsoidal oocyst; usual range, 20–30 µm long by 10–19 µm wide; sporocysts rarely seen broken out of oocysts but measure 9–11 µm	Mature oocyst contains 2 sporocysts with 4 sporozoites each; the usual diagnostic stage in feces is the immature oocyst, containing a spherical mass of protoplasm (diarrheic stool). Developing stages can be recovered from intestinal biopsy specimens. Oocysts are also acid fast and could be detected during acid-fast staining of stool for *Cryptosporidium*. Oocysts are often detected in the concentration sediment (wet preparation); they are not immediately infectious when passed.

(continued)

TABLE A1.13 *(continued)*

Species	Shape and size	Other features[b]
Microsporidia[c] *Nosema* spp. *Encephalitozoon* spp. *Enterocytozoon* spp. *Pleistophora* spp. *Trachipleistophora* spp. *Anncaliia* spp. *Microsporidium* spp. *Vittaforma corneae* *Tubulinosema* sp.	Spores are extremely small (1.5–2.5 μm) and have been recovered in all body organs, including the eye	These organisms have been found as insect or other animal parasites; the route of infection may be ingestion, inhalation, or direct inoculation (eyes). Histology results vary (spores are acid fast); PAS, silver, tissue Gram, and Giemsa stains are recommended for spores. Animal inoculation is not recommended; laboratory animals may carry occult infection. Electron microscopy or molecular methods may be necessary for confirmation and identification to the genus and species levels. Although difficult to diagnose, infections have been found in a large number of AIDS patients (*Enterocytozoon bieneusi*, *Encephalitozoon* [*Septata*] *intestinalis* in the intestinal tract, *Pleistophora* spp. in muscle, and various other microsporidia in other tissues). To date, it is still somewhat difficult to diagnose this infection by examining stool specimens prepared with optical brightening agents (calcofluor) or routine stains (modified trichrome stains). Diagnostic immunoassay reagents are under development.
Sarcocystis hominis, *S. suihominis*, *S. bovihominis*	Oocyst is thin walled and contains 2 mature sporocysts, each containing 4 sporozoites; the thin oocyst wall frequently ruptures; ovoidal sporocysts measure 9–16 μm long by 7.5–12 μm wide	Thin-walled oocyst or ovoidal sporocysts are found in stool. There have been reports of fever, severe diarrhea, abdominal pain, and weight loss in the compromised host, although the number of patients has been small. Infections occur from the ingestion of uncooked pork or beef. The life cycle occurs within the intestinal cells, with the eventual production of sporocysts in stool.
Sarcocystis "*lindemanni*" (this name is no longer used)	Shapes and sizes of skeletal and cardiac muscle sarcocysts vary considerably	When humans accidentally ingest oocysts from other animal stool sources, sarcocysts that develop in human muscle apparently do little if any harm. These could be identified by routine histologic methods. When humans are intermediate hosts, preventive measures involve careful disposal of animal feces that may contain the infective sporocysts. This may be impossible in the wilderness areas, where wild animals may serve as reservoir hosts for many of the different types of organisms that have been grouped under the term *Sarcocystis* "*lindemanni*." However, this name is no longer used.

[a] Modified from Garcia LS, ed, *Clinical Microbiology Procedures Handbook,* 3rd ed, ASM Press, Washington, DC, 2010.

[b] CNS, central nervous system; CSF, cerebrospinal fluid; PAS, periodic acid-Schiff.

[c] Now classified with the fungi.

TABLE A1.14 Key characteristics of helminths[a]

Helminths	Diagnostic stage[b]	Comments
Nematodes (roundworms)		
Ascaris lumbricoides (pathogenic)	Egg: both fertilized (oval to round with thick, mammilated/tuberculated shell) and unfertilized (tend to be more oval or elongate, with bumpy shell exaggerated) eggs can be found in stool; adult worms: 10–12 in. found in stool; rarely (in severe infections), migrating larvae can be found in sputum.	Unfertilized eggs do not float in flotation concentration method; adult worms tend to migrate when irritated (by anesthesia or high fever); hence, patients from areas of endemic infection should be checked for infection prior to elective surgery.
Trichuris trichiura (whipworm) (pathogenic)	Egg: barrel shaped with 2 clear, polar plugs; adult worm: rarely seen; eggs should be quantitated (rare, few, etc.), since light infections may not be treated.	Dual infections with *A. lumbricoides* may be seen (both infections are acquired from ingestion of eggs from contaminated soil); in severe infections, rectal prolapse may occur in children or bloody diarrhea can be mistaken for amebiasis (these clinical manifestations are usually not seen in the United States).
Enterobius vermicularis (pinworm)	Egg: football shaped with one flattened side; adult worm: about 3/8 in. long, white with pointed tail; female migrates from the anus and deposits eggs on the perianal skin.	Causes itching in some patients. Test of choice is Scotch tape preparation; 4–6 consecutive tapes necessary to rule out infection; symptomatic patients often treated without actual confirmation of infection; eggs become infective within a few hours.
Ancylostoma duodenale (Old World hookworm), *Necator americanus* (New World hookworm) (pathogenic)	Egg: eggs of both species are identical, oval with broadly rounded ends, thin shell, clear space between shell and developing embryo (8–16-cell stage); adult worms: rarely seen in clinical specimens	Causes blood-loss anemia on the differential smear in heavy infections in some patients. If stool remains unpreserved for several hours or days, the eggs may continue to develop and hatch; rhabditiform larvae may resemble those of *Strongyloides stercoralis*.
Strongyloides stercoralis (pathogenic)	Rhabditiform larvae (noninfective) usually found in the stool; short buccal cavity or capsule with large genital primordial packet of cells ("short and sexy"); in very heavy infections, larvae occasionally found in sputum and/or filariform (infective) larvae can be found in stool (slit in the tail)	May cause unexplained eosinophilia, abdominal pain, unexplained episodes of sepsis and/or meningitis, and pneumonia (migrating larvae) in the compromised patient. Potential for internal autoinfection can maintain low-level infections for many years (patient is asymptomatic or has eosinophilia); hyperinfection can occur in the compromised patient (leading to disseminated strongyloidiasis and death); agar plate culture is the most sensitive diagnostic method; many infections are low-level, and larvae are difficult to recover.
Ancylostoma braziliensis (dog or cat hookworm) (pathogenic)	Humans are accidental hosts; larvae wander through the outer layer of skin, creating tracks (causing severe itching and eosinophilia); no practical microbiological diagnostic tests	Cause of cutaneous larva migrans; typical setup for infection is when dogs and cats defecate in sand boxes and hookworm eggs hatch and penetrate human skin when in contact with infected sand or soil (children playing in sand box).
Toxocara cati and *T. canis* (dog and cat ascarid) (pathogenic)	Humans are accidental hosts; infection is by ingestion of dog or cat ascarid eggs in contaminated soil; larvae wander through deep tissues (including the eye); can be mistaken for cancer of the eye; serologic tests helpful for confirmation; infection causes eosinophilia.	Cause of visceral larva migrans, ocular larva migrans, and neural larva migrans; requests for laboratory services often originate in the ophthalmology clinic.
Cestodes (tapeworms)		
Taenia saginata (beef tapeworm)	Scolex (4 suckers, no hooklets) and gravid proglottid (>12 branches on a single side) are diagnostic; eggs indicate *Taenia* spp. only (thick, striated shell containing a 6-hooked embryo or oncosphere); worm usually about 12–15 ft long.	Adult worm causes symptoms in some individuals. Infection occurs via ingestion of raw or poorly cooked beef; usually only a single worm per patient; individual proglottids may crawl from the anus; India ink can be injected into proglottids to visualize the uterine branches for identification.
Taenia solium (pork tapeworm)	Scolex (4 suckers with hooklets) and gravid proglottid (<12 branches on a single side) are diagnostic; eggs indicate *Taenia* spp. only (thick, striated shell containing a 6-hooked embryo or oncosphere); worm is usually about 6–20 ft long.	Adult worm causes gastrointestinal complaints in some individuals; cysticercosis (accidental ingestion of eggs) can cause severe central nervous system symptoms. Infection is via ingestion of raw or poorly cooked pork; usually only a single worm per patient; occasionally 2 or 3 proglottids (hooked together) are passed. India ink can be injected into proglottids to visualize the uterine branches for identification. Cysticerci are normally small and contained within an enclosing membrane; they occasionally develop as the "racemose" type in which the worm tissue grows in the body like a metastatic cancer.

Diphyllobothrium latum (broad fish tapeworm)	Scolex (lateral sucking grooves), gravid proglottid (wider than long, reproductive structures in the center "rosette"); eggs are operculated.	Causes gastrointestinal complaints in some individuals. Infection is via ingestion of raw or poorly cooked freshwater fish; life cycle has 2 intermediate hosts (copepod and fish); worm may reach 30 ft long. Associated with vitamin B_{12} deficiency in genetically susceptible groups (e.g., Scandinavians).
Hymenolepis nana (dwarf tapeworm)	Adult worm not normally seen; egg round to oval, thin shell, containing a 6-hooked embryo or oncosphere with polar filaments lying between the embryo and egg shell.	Causes gastrointestinal complaints in some individuals. Infection is via ingestion of eggs (the only life cycle in which the intermediate host [grain beetle] can be bypassed); life cycle of egg to larval form to adult can be completed in the human. May be the most common tapeworm in the world.
Hymenolepis diminuta (rat tapeworm)	Adult worm not normally seen; egg round to oval, thin shell, containing a 6-hooked embryo or oncosphere without polar filaments lying between the embryo and egg shell.	Uncommon; egg can be confused with *H. nana*. Eggs will be submitted in proficiency-testing specimens and must be differentiated from those of *H. nana*.
Echinococcus granulosus (pathogenic)	Adult worm found only in the carnivore (dog); hydatid cysts develop (primarily in the liver) when humans accidentally ingest eggs of the dog tapeworms; cyst contains daughter cysts and many scolices; laboratory should examine fluid aspirated from cyst at surgery.	Humans are accidental intermediate hosts; the normal life cycle is dog to sheep, with the hydatid cysts developing in the liver, lung, etc., of the sheep. Human hosts may be unaware of their infection unless fluid leaks from the cyst (can trigger an anaphylactic reaction) or pain is felt at the cyst location.
Echinococcus multilocularis (pathogenic)	Adult worm found only in the carnivore (fox or wolf); hydatid cysts develop (primarily in the liver) when humans accidentally ingest eggs of the carnivore tapeworms; cyst grows like a metastatic cancer with no limiting membrane.	Humans are accidental intermediate hosts. Prognosis is poor; surgical removal of the tapeworm tissue is very difficult. Found in Canada, Alaska, and less frequently in the northern United States but is becoming more common in the United States, where the geographic range is moving further south.
Trematodes (flukes)		
Fasciolopsis buski (giant intestinal fluke)	Eggs found in stool; very large and operculated (morphology like that of *F. hepatica* eggs)	Symptoms depend on worm burden; acquired from ingestion of plant material on which metacercariae have encysted (e.g., water chestnuts); worms are hermaphroditic.
Fasciola hepatica (sheep liver fluke)	Eggs found in stool; cannot be differentiated from those of *F. buski*	Symptoms depend on worm burden; acquired from ingestion of plant material on which metacercariae have encysted (e.g., watercress); worms are hermaphroditic.
Clonorchis (*Opisthorchis*) *sinensis* (Chinese liver fluke)	Eggs found in stool; very small (<35 µm); are operculated with shoulders into which the operculum fits	Symptoms depend on worm burden; acquired from ingestion of raw fish. Eggs can be missed unless ×400 power is used for examination; eggs can resemble those of *Metagonimus yokogawai* and *Heterophyes heterophyes* (small intestinal flukes). Worms are hermaphroditic.
Paragonimus spp. (lung fluke)	Eggs coughed up in sputum (brownish "iron filings" are egg packets); can be recovered in sputum or stool (if swallowed); are operculated with shoulders into which operculum fits	Symptoms depend on worm burden and egg deposition. Infection acquired from ingestion of raw crabs. Eggs can be confused with those of *D. latum*. Infections seen in Asia (infections with *P. mexicanus* found in Central and South America). *P. kellicotti* infections (rare) are seen in the United States. Worms are hermaphroditic but often cross-fertilize with another worm if present.
Schistosoma mansoni (blood fluke)	Eggs recovered in stool (large lateral spine); specimens should be collected with no preservatives (to allow demonstration of egg viability); worms in veins of large intestine	Acquired from skin penetration by a single cercaria from the freshwater snail. Pathologic findings are caused by host immune response to the presence of eggs in tissues; adult worms in veins cause no problems. Adult worms are separate sexes.
Schistosoma haematobium (blood fluke)	Eggs recovered in urine (large terminal spine); specimens should be collected with no preservatives (to allow demonstration of egg viability); worms in veins of bladder	Acquired from skin penetration by single cercaria from the freshwater snail; pathologic findings as with *S. mansoni;* 24-h and spot urine samples should be collected. Chronic infection has association with bladder cancer. Adult worms are separate sexes.
Schistosoma japonicum (blood fluke)	Eggs recovered in stool (very small lateral spine); specimens should be collected with no preservatives (to allow demonstration of egg viability); worms in veins of small intestine	Acquired from skin penetration by multiple cercariae from the freshwater snail; pathologic findings as with *S. mansoni;* infection usually the most severe of the 3 species because of original loading dose of infective cercariae from the freshwater snail (multiple cercariae stick together). Symptoms are associated with egg production, which is greatest in *S. japonicum* infections.

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[a] Modified from Garcia LS, ed, *Clinical Microbiology Procedures Handbook*, 3rd ed, ASM Press, Washington, DC, 2010.

[b] 1 in. = 2.54 cm; 1 ft = 30.48 cm.

TABLE A1.15 Key characteristics of most common parasites found in blood[a]

Organism	Diagnostic stage	Comments
Protozoa		
Malaria parasites		
Plasmodium vivax (benign tertian malaria)	Ameboid rings; presence of Schüffner's dots, beginning in older rings (appear later than those in *P. ovale*); all stages seen in peripheral blood; mature schizont contains 16–18 merozoites.	Infects young cells; 48-h cycle; large geographic range; tends to have true relapse from the residual liver stages (hypnozoites); enlarged RBCs
Plasmodium ovale (ovale malaria)	Nonameboid rings; presence of Schüffner's dots, beginning in young rings (appear earlier than those in *P. vivax*); all stages seen in peripheral blood; mature schizont contains 8–10 merozoites; RBCs may be oval and have fimbriated edges.	Infects young cells; 48-h cycle; narrow geographic range; tends to have true relapse from residual liver stages (hypnozoites); enlarged RBCs
Plasmodium malariae (quartan malaria)	Thick rings; no stippling; all stages seen in peripheral blood; presence of band forms and rosette-shaped mature schizont; abundant malarial pigment	Infects old cells; 72-h cycle; narrow geographic range; associated with recrudescence and nephrotic syndrome; no true relapse; normal or small RBCs
Plasmodium falciparum (malignant tertian malaria)	Multiple rings; appliqué/accolé forms; no stippling (rare Maurer's clefts); rings and crescent-shaped gametocytes seen in peripheral blood (no other developing stages, with rare exception of mature schizont)	Infects all cells; 36–48-h cycle; large geographic range; no true relapse; most pathogenic of the 4 species; plugged capillaries can cause severe symptoms and sequelae (cerebral malaria, lysis of RBCs, etc.).
Plasmodium knowlesi (simian malaria)	Multiple rings; no stippling; all stages seen in peripheral blood; will mimic *P. falciparum* early in the life cycle, then will mimic *P. malariae* in the later stages; often identified as a mixed infection with those two species	Infects all cells; very rapid cycle of 24 h; small geographic range (Malaysia and neighboring areas); as pathogenic as *P. falciparum;* development in RBCs may be asynchronous; no true relapse
Babesia spp.	Ring forms only (resemble *P. falciparum* rings); seen in splenectomized patients; endemic in the United States (no travel history necessary); if present, Maltese cross configuration is diagnostic but not always seen (more common in West Coast organisms; less common in East Coast, *B. microti*	Tick-borne infection; associated with Nantucket Island; infection mimics malaria; ring forms more pleomorphic than malaria; usually more rings/cell than in malaria; endemic in several areas in the United States (East and West Coasts); organisms occasionally seen outside RBCs (unlike malaria merozoites)
Trypanosomes		
Trypanosoma brucei gambiense (West African sleeping sickness)	Trypomastigotes long, slender, with typical undulating membrane and very tiny kinetoplast; lymph nodes and blood can be sampled; microhematocrit tube concentration helpful; examine spinal fluid in later stages of the infection; elevated IgM	Tsetse fly vector; tends to be chronic infection, causing the real symptoms of sleeping sickness; more chronic infection; division occurs in the trypomastigote stage.
Trypanosoma brucei rhodesiense (East African sleeping sickness)	Trypomastigotes long, slender, with typical undulating membrane; small kinetoplast; lymph nodes and blood can be sampled; microhematocrit tube concentration helpful; examine spinal fluid in later stages of the infection; elevated IgM	Tsetse fly vector; tends to be more severe, short-lived infection (particularly in children); patient may die before progressive symptoms of sleeping sickness appear; division occurs in the trypomastigote stage.
Trypanosoma cruzi (Chagas' disease) (American trypanosomiasis)	Trypomastigotes short, stumpy, often curved in C shape; large kinetoplast; blood sampled early in infection; trypomastigotes enter striated muscle (heart, gastrointestinal tract) and transform into the amastigote form.	Reduviid bug vector (kissing bug); chronic in adults but severe in young children; great morbidity associated with cardiac failure and loss of muscle contractility in heart and gastrointestinal tract; division occurs in the amastigote stage.

Trypanosoma rangeli	Trypomastigotes long, slender, with typical undulating membrane and tiny kinetoplast	Reduviid bug vector; *T. rangeli* infections often overlap with *T. cruzi* infections; in some areas, *T. rangeli* represents 5 or 6 times the number of *T. cruzi* infections; assumed to be nonpathogenic for humans; has been noted in the blood for >1 year; infections with *T. cruzi* and *T. rangeli* can coexist in the reduviid vector.
Leishmaniae		
Leishmania spp. (cutaneous; not actually a blood parasite but presented for comparison with *L. donovani*)	Amastigotes found in macrophages of skin; presence of intracellular forms containing nucleus and bar-shaped kinetoplast is diagnostic.	Sand fly vector; organisms recovered from site of lesion only; specimens can be stained or cultured in NNN[b] and/or Schneider's medium; animal (hamster) inoculation rarely used
Leishmania braziliensis and other *Leishmania* spp. (mucocutaneous; not actually a blood parasite but presented for comparison with *L. donovani*)	Amastigotes found in macrophages of skin and mucous membranes; presence of intracellular forms containing nucleus and bar-shaped kinetoplast is diagnostic.	Sand fly vector; organisms recovered from site of lesion only; specimens can be stained or cultured in NNN and/or Schneider's medium; animal (hamster) inoculation rarely used
Leishmania donovani and other *Leishmania* spp. (visceral)	Amastigotes found throughout the reticuloendothelial system (spleen, liver, bone marrow, etc.); presence of intracellular forms containing nucleus and bar-shaped kinetoplast diagnostic; patients have hypergammaglobulinemia	Sand fly vector; organisms recovered from buffy coat monocytes (rarely found), bone marrow aspirate, spleen or liver puncture (rarely performed); specimens can be stained or cultured in NNN and/or Schneider's medium; animal (hamster) inoculation rarely used; cause of kala azar
Helminths		
Wuchereria bancrofti	Microfilariae sheathed, clear space at end of tail; nocturnal periodicity seen; elephantiasis seen in chronic infections	Pathogenicity due to presence of adult worms; mosquito vector; microfilariae recovered in blood (membrane filtration, Knott concentrate, thick films); hematoxylin stains the sheath; Giemsa stain does not stain the sheath.
Brugia malayi	Microfilariae sheathed, subterminal and terminal nuclei at end of tail; nocturnal periodicity seen; elephantiasis seen in chronic infections	Pathogenicity due to presence of adult worms; mosquito vector; microfilariae recovered in blood (membrane filtration, Knott concentrate, thick films); hematoxylin stains the sheath; Giemsa stains the sheath pink; sheath of *B. timori* does not stain with Giemsa.
Loa loa (African eye worm)	Microfilariae sheathed, nuclei continuous to tip of tail; diurnal periodicity; adult worm may cross the conjunctiva of the eye.	Pathogenicity due to presence of adult worms; mango fly vector; history of calabar swellings, worms in the eye; microfilariae difficult to recover from blood; hematoxylin stains the sheath; Giemsa stain does not stain the sheath.
Mansonella spp.	Microfilariae unsheathed, nuclei may or may not extend to tip of tail (depending on species); nonperiodic; symptoms usually absent or mild	Pathogenicity mild and due to presence of adult worms; midge or blackfly vector; microfilariae recovered in blood (membrane filtration, Knott concentrate, thick films); hematoxylin and Giemsa stain characteristics identical
Mansonella streptocerca	Microfilariae unsheathed, nuclei extend to tip of tail; when immobile, curved like shepherd's crook; adults in dermal tissues	Pathogenicity mild and due to presence of adult worms and/or microfilariae; midge vector; microfilariae found in skin snips; microfilarial tails are split rather than blunt; hematoxylin and Giemsa stain characteristics identical
Onchocerca volvulus	Microfilariae unsheathed, nuclei do not extend to tip of tail; adults in nodules	Pathogenicity due to presence of microfilariae; blackfly vector; microfilariae found in skin snips; microfilariae migrate to optic nerve; cause of river blindness

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[a] Modified from Garcia LS, ed, *Clinical Microbiology Procedures Handbook*, 3rd ed, ASM Press, Washington, DC, 2010. NNN, Novy-MacNeal-Nicolle; RBC, red blood cell.

TABLE A1.16 Diagnostic laboratory report information that should be relayed to the physician[a]

Organisms and other findings	Genus and species	Life cycle stages	Quantitation[b]	Pathogenic/ nonpathogenic	Viable/nonviable
Protozoa					
Intestinal	Yes	Yes (C, T)	No	Both	NA[c]
Blastocystis spp.		NA	Yes	Pathogenicity comment	NA
Tissue	Yes	NA	No	NA	NA
Blood	Yes	Yes (G)	Yes	NA	NA
Genitourinary tract	Yes	NA	No	NA	NA
Trematodes					
Intestinal	Yes	NA	Yes	NA	NA
Liver	Yes	NA	Yes	NA	NA
Lungs	Yes	NA	Yes	NA	NA
Blood	Yes	NA	Yes	NA	Egg viability
Cestodes					
Intestinal	Yes	Yes (E, P)	NA	NA	NA
Tissue	Yes	NA	NA	NA	NA
Nematodes					
Intestinal	Yes	NA	Rarely[d]	NA	NA
Filarial	Yes	NA	Yes	NA	NA
Nonparasitic					
Yeast	NA	Yes[e]	Yes	NA	NA
Human cells (WBCs, RBCs)	NA	NA	Yes	NA	NA
Crystals (C-L, barium)	NA	NA	Yes	NA	NA

[a] C, cyst; C-L, Charcot-Leyden; E, egg; G, gametocyte; P, proglottid; RBC, red blood cell; T, trophozoite; WBC, white blood cell.

[b] May require numbers of infected cells, eggs, etc. (either as specific count or as rare, few, moderate, or many).

[c] NA, not applicable.

[d] If *Trichuris trichiura* eggs are rare or few, the infection may not be treated.

[e] The specimen must be fresh and should be examined immediately; alternatively, it should be freshly preserved in a stool collection kit *by the patient* (no lag time between stool passage and preservation); if these requirements are not met, the report (presence of budding yeasts and/or pseudohyphae or hyphae) may be misleading and should not be reported to the physician. Report budding yeast cells and/or the presence of pseudohyphae or hyphae as a consult with physician (not routinely reported; may be misleading).

TABLE A1.17 Pros and cons of stool specimen collection and testing options[a]

Option[b]	Pros	Cons
Rejection of stools from inpatients who have been in-house for >3 days	Data suggest that patients who begin to have diarrhea after they have been inpatients for a few days are not symptomatic from parasitic infections, but generally from other causes.	There is always the chance that the problem is related to a health care-associated (nosocomial) parasitic infection (rare), but *Cryptosporidium* and microsporidia are possible considerations.
Examination of 1 stool (O&P examination)	Data suggest that 40–50% of organisms present will be found with only 1 stool exam. Some feel that most intestinal parasitic infections can be diagnosed from examination of a single stool. If the patient becomes asymptomatic after collection of the first stool, subsequent specimens may not be necessary.	Diagnosis from examination of a single specimen depends on the experience of the microscopist, proper collection, and the parasite load in the specimen. In a series of 3 stool specimens, frequently not all 3 specimens are positive and/or may be positive for different organisms.
Use of 2 O&P exams (concentration, permanent stained smear)	The examination of 2 specimens is more sensitive than 1 examination; different organisms may be found in the 2 specimens; 1 might be negative while 1 is positive.	May not be as cost-effective as examination of 1 specimen. Not always as good as examination of 3 specimens (may be a relatively cost-effective approach); any patient remaining symptomatic would require additional testing.
Examination of a second stool specimen only after the first is negative and the patient is still symptomatic	With additional examinations, the yield of protozoa increases (*Entamoeba histolytica*, 22.7%; *Giardia lamblia*, 11.3%; *Dientamoeba fragilis*, 31.1%)	Assumes that the second (or third) stool is collected within the recommended 10-day time frame for a series of stools; protozoa are shed periodically. May be inconvenient for patient.
Examination of 1 stool specimen and an immunoassay (EIA, FA, lateral- or vertical-flow cartridge)	If the examinations are negative and the patient's symptoms subside, probably no further testing is required.	This approach is a mix: 1 immunoassay may be acceptable; however, immunoassay testing of 2 separate specimens may be required to confirm the presence of *Giardia* antigen. One O&P exam is not the best approach (review the last option below). Patients may exhibit symptoms (off and on), so it may be difficult to rule out parasitic infections. If the patient remains symptomatic, even if 2 *Giardia* immunoassays are negative, other protozoa may be missed (the *Entamoeba histolytica/E. dispar* group, *E. histolytica*, *Dientamoeba fragilis*, *Cryptosporidium* spp., the microsporidia). Normally, there are specific situations where fecal immunoassays or O&P exams should be ordered. It is not recommended to automatically perform both the O&P and fecal immunoassay as a stool exam for parasites (see Table A1.18). **Depending on the patient's history and clinical symptoms, EITHER the O&P exam OR a fecal immunoassay may be recommended, BUT GENERALLY NOT BOTH.**
Pool 3 specimens for examination; perform 1 concentration and 1 permanent stain	Three specimens are collected by the patient (3 separate collection vials) over 7–10 days; pooling by the laboratory may save time and expense.	Organisms present in small numbers may be missed due to the dilution factor once the specimens are pooled.
Pool 3 specimens for examination; perform 1 concentration and 3 permanent stained smears	Three specimens are collected by the patient (3 separate collection vials) over 7–10 days; pooling by the laboratory for the concentration would probably be sufficient for the identification of helminth eggs. Examination of the 3 separate permanent stained smears (1 from each vial) would maximize recovery of intestinal protozoa in areas of the country where these organisms are most common.	Might miss light helminth infection (eggs, larvae) due to the pooling of the 3 specimens for the concentration; however, with a permanent stain performed on each of the 3 specimens, this approach would probably be the next best option to the standard approach (concentration and permanent stained smear performed on every stool). Coding and billing would have to match the work performed; this may present some problems where work performed does not match existing codes and billing. Per standard recommendations and CAP, O&P must include the concentration and permanent stained smear.

(continued)

TABLE A1.17 Pros and cons of stool specimen collection and testing options[a] *(continued)*

Option[b]	Pros	Cons
Collection of 3 stools by the patient, with all 3 samples put into a single vial (patient given a single vial only)	Pooling of the specimens would require only a single vial.	This would complicate patient collection and very likely result in poorly preserved specimens, especially regarding the recommended ratio of stool to preservative and the lack of proper mixing of specimen and fixative.
Immunoassays performed for selected patients,[c] using methods for *G. lamblia*, *Cryptosporidium* spp., and/or the *E. histolytica/E. dispar* group or *E. histolytica*	Would be more cost-effective than performing immunoassay procedures on all specimens; however, information required to group patients is often not received with specimens. *This approach assumes that the physicians have guidance in terms of correct ordering options (see Table A1.18).*	Laboratories rarely receive information that would allow them to place a patient in a particular risk group: children <5 yr old, children from day care centers (may or may not be symptomatic), patients with immunodeficiencies, and patients from outbreaks. Performance of immunoassay procedures on every stool sample is not cost-effective, and the positive rate will be low unless an outbreak situation is involved.
Immunoassays and O&P exams performed on request using methods for *G. lamblia*, *Cryptosporidium* spp., and/or *E. histolytica/E. dispar* group or *E. histolytica* (a number of variables will determine the approach to immunoassay testing and the O&P exam [geography, parasites recovered, positive rate, physician requests]; immunoassays and/or O&P exams should be separately ordered, reported, and billed)	This approach limits the number of stools on which immunoassay procedures are performed for parasites. Immunoassay results do not have to be confirmed by any other tests (such as O&P exams or modified acid-fast stains). If specific kit performance problems have been identified, individual laboratories may prefer to do additional testing. *However, the fecal immunoassays are more sensitive than the O&P exam and special stains (modified acid-fast stains).* Also, this may be considered duplicate testing and may not be approved for reimbursement unless specifically ordered by the physician.	Requires education of the physician clients regarding appropriate times and patients for whom fecal immunoassays should be ordered. Educational initiatives must also include information on the test report indicating the pathogenic parasites that will not be detected by these methods. It is critical to ensure that clients know that if patients have become asymptomatic, further testing may not be required. *However, if the patient remains symptomatic, further testing (O&P exams) is required*. Remember, a single O&P exam may not reveal all organisms present. Present plan to physicians for approval: immunoassays or O&P exams, procedure discussion, report formats, clinical relevance, and limitations on each approach.

[a] For further information, see the following papers: Hiatt RA, et al, *Am J Trop Med Hyg* **53**:36–39, 1995; Kehl KSC, *Clin Microbiol Newsl* **18**:133–135, 1996; Morris AJ, et al, *J Clin Microbiol* **30**:3213–3216, 1992; Siegel DL, et al, *JAMA* **263**:979–982, 1990.

[b] O&P exam, ova and parasite examination; EIA, enzyme-linked immunoassay; FA, fluorescent-antibody immunoassay; CAP, College of American Pathologists.

[c] It is difficult to know when you may be in an early-outbreak situation where testing of all specimens for either *Giardia lamblia* or *Cryptosporidium* spp., or both, may be relevant. Extensive efforts are under way to encourage communication among laboratories, water companies, pharmacies, and public health officials regarding the identification of potential or actual outbreaks. If it appears that an outbreak is in the early stages, then performing the immunoassays on request can be changed to screening all stools.

TABLE A1.18 Approaches to stool parasitology: test ordering

Patient and/or situation	**Test ordered**[a]	**Follow-up test ordered**
♦ Patient with diarrhea and AIDS or other cause of immune deficiency ♦ Potential waterborne outbreak (municipal/city water supply)	*Cryptosporidium* or *Giardia/Cryptosporidium* immunoassay	If immunoassays are negative and symptoms continue, special tests for microsporidia (modified trichrome stain) and other coccidia (modified acid-fast stain) and O&P exam should be performed.
♦ Patient with diarrhea (nursery school, day care center, camper, backpacker) ♦ Patient with diarrhea and potential waterborne outbreak (resort setting) ♦ Patient with diarrhea from areas where *Giardia* is the most common parasite found	*Giardia* or *Giardia/Cryptosporidium* immunoassay (perform testing on two stools before reporting as negative) **Particularly relevant for areas of the U.S. where *Giardia* is the most common organism found**	If immunoassays are negative and symptoms continue, special tests for microsporidia and other coccidia (see above) and O&P exam should be performed.
♦ Patient with diarrhea and relevant travel history outside the United States ♦ Patient with diarrhea who is a past or present resident of a developing country ♦ Patient in an area of the United States where parasites **other than *Giardia*** are found (large metropolitan areas like Los Angeles, New York, Boston, Miami, etc.)	O&P exam,[b] *Entamoeba histolytica/E. dispar* immunoassay; immunoassay for confirmation of *E. histolytica*; various tests for *Strongyloides* may be relevant (even in the absence of eosinophilia), particularly if there is any history of pneumonia (migrating larvae in lungs), sepsis, or meningitis (fecal bacteria carried by migrating larvae); agar culture plate is the most sensitive diagnostic approach for *Strongyloides*.	The O&P exam is designed to detect and identify a broad range of parasites (amebae, flagellates, ciliates, *Cystoisospora belli*, helminths); if exams are negative and symptoms continue, special tests for coccidia (fecal immunoassays, modified acid-fast stains, autofluorescence) and microsporidia (modified trichrome stains, calcofluor white stains) should be performed; fluorescent stains are also options.
♦ Patient with unexplained eosinophilia and possible diarrhea; if chronic, patient may also have history of respiratory problems (larval migration) and/or sepsis or meningitis (hyperinfection)	Although the O&P exam is recommended, the agar plate culture for *Strongyloides stercoralis* (more sensitive than the O&P exam) is also recommended, particularly if there is any history of pneumonia (migrating larvae in lungs), sepsis, or meningitis (fecal bacteria carried by migrating larvae).	If tests are negative and symptoms continue, additional O&P exams and special tests for microsporidia (modified trichrome stains, calcofluor white stains, fluorescent stains) and other coccidia (modified acid-fast stains, autofluorescence, fluorescent stains) should be performed; serology for *Strongyloides* may also be recommended.
♦ Patient with diarrhea (suspected food-borne outbreak)	Test for *Cyclospora cayetanensis* (modified acid-fast stain, autofluorescence, fluorescent stains)	If tests are negative and symptoms continue, special procedures for microsporidia and other coccidia and O&P exam should be performed.

[a] Depending on the particular immunoassay kit used, various single or multiple organisms may be included. Selection of a particular kit depends on many variables: clinical relevance, cost, ease of performance, training, personnel availability, number of test orders, training of physician clients, sensitivity, specificity, equipment, time to result, etc. Very few laboratories will handle this type of testing exactly the same. Many options are clinically relevant and acceptable for good patient care. It is critical that the laboratory report indicate specifically which organisms could be identified using the kit; a negative report should list the organisms relevant to that particular kit. It is important to remember that sensitivity and specificity data for all of these fecal immunoassay kits (fluorescent-antibody assay, enzyme immunoassay, cartridge formats) are comparable.

[b] O&P exam, ova and parasite examination.

TABLE A1.19 Pros and cons of ova and parasite examination options[a]

Testing option[b]	Pros	Cons
Stools from patients who have been inpatients for at least 3 days should not be submitted for routine examinations without consulting the laboratory.	Normally, only very few parasitic infections can be acquired by an inpatient. Although rare, one of these infections is cryptosporidiosis, which has been documented in nosocomial transmission.	Etiologic agents other than parasites, as well as other causes such as therapy, are more likely to cause diarrhea in patients who have been hospitalized for 3 days or more. There are always exceptions, but this is a reasonable guideline to follow (see Table A1.17).
3 separate stools collected within 10 days; concentration and permanent stained smear on all specimens; if specimen is fresh (unpreserved), direct wet smear can be performed (soft, liquid specimens) for the detection of organism motility	Maximize recovery of all organisms, including those in small numbers; concentration for recovery of protozoan cysts, helminth eggs, and larvae; permanent stain for confirmation of protozoa and finding protozoa missed on concentration wet examination. Special stains or immunoassay reagents may be used for the recovery of coccidia and microsporidia.	Although this approach is the most labor intensive, the results are the most complete.[c] According to recommendations (CLSI, Cumitechs, College of American Pathologists Microbiology Checklist) an O&P must include the concentration and permanent stain to be coded/billed as an O&P examination.
3 separate stools collected within 10 days; concentration on pooled specimen, permanent stained smear on all specimens (performed as 3 separate examinations)	By pooling 3 specimens and performing one concentration and examination, time is saved. Since intestinal protozoa tend to be the most common parasites found in many areas of the United States, it is important to perform permanent stained smears on the 3 individual specimens.	If helminth eggs or larvae are present in small numbers, they may be missed. Protozoa that may be missed in small numbers will probably be identified on the individual permanent stained smears.
1 stool; concentration and permanent stained smear; additional specimens may be examined after results of the first specimen are reported	Saves time	Organisms present in small numbers may be missed; the collection of specimens 2 or 3 may be outside the 10-day recommended time frame. The series of 3 specimens now becomes 3 series of a single specimen (depending on collection time frames).[c]
1 stool; *Giardia* and *Cryptosporidium* immunoassay[d]	This can be ordered after complete discussion with physicians so that they recognize what the results (negative) mean in terms of limitations; if negative with a patient who continues to be symptomatic, ova and parasite examinations can be ordered. In areas where *Giardia lamblia* is commonly found or in a suspect waterborne outbreak (*Cryptosporidium* or other organisms), this is an acceptable option. However, it is now clear that 2 specimens may have to be examined to confirm the presence of giardiasis (this is not applicable for cryptosporidiosis).	If complete information on the relevance of a negative report is not given to the physician, this approach can be used incorrectly. It is very important that immunoassay procedures be thoroughly discussed in terms of the clinical relevance of results and the specificity and sensitivity of the methods (more sensitive than the routine ova and parasite examination).
1 stool; concentration performed; permanent stained smear performed *only* if something suspicious is seen on the concentration examination	This approach is thought to save time; however, it is unacceptable both technically and in terms of quality patient care.	Numerous studies have confirmed the importance of the permanent stain, not only in confirming the identification of intestinal protozoa *but also in finding organisms that were missed in the concentration examination*. Use of this technical approach leads to very misleading reported results and should be discouraged. If a laboratory is using this approach, the specimens for parasitology testing should be sent to another laboratory.

Examination approach Direct wet smear: unpreserved soft or liquid stools; total 22- by 22-mm coverslip with 10× objective (low power); 1/4–1/3 of area with 40× objective (high dry power) Concentration: total 22- by 22-mm coverslip with 10× objective (low power); 1/4–1/3 of area with 40× objective (high dry power) Permanent stained smear: minimum of 300 fields with 100× objective (oil immersion); a 50× or 60× oil immersion lens can also be used in the examination process, but some small organisms will be missed without the use of the 100× oil immersion lens.	This represents the most complete approach; however, the important point is to understand the pros and cons of any modifications to this approach. The most common change is the transition from fresh, unpreserved specimens being submitted to the laboratory to fecal specimens that are transported in fixatives. Once received by the laboratory, the direct wet smear is eliminated from the standard ova and parasite procedure (no motility is visible in preserved specimens) and only the concentration and permanent stained smear are performed.	Any modification(s) from the recommended procedures *must* be discussed thoroughly with your physician clients. It is critical that they understand the advantages and disadvantages of each approach. Information can be transmitted through various communication channels: telephone, newsletters, information updates, in-service presentations, verbal and written reminders, information via computer system, etc.

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[a] **It is mandatory that the physicians know exactly what procedures are performed for the diagnosis of parasitic infections in your specific laboratory and how the results should be interpreted in terms of clinical relevance. It is mandatory that they be given information regarding the pros and cons (limitations) of any diagnostic approach. The complete ova and parasite examination includes direct wet smear, concentration, and permanent stained smear (fresh specimen) or concentration and permanent stained smear (preserved specimen).**

[b] A direct wet smear of fresh specimens (soft or liquid) should be examined for motile trophozoites. If the specimen is formed or is received in preservative, eliminate the direct wet smear examination and proceed directly to the concentration and permanent stained smear.

[c] An excellent reference is Hiatt RA, et al, *Am J Trop Med Hyg* 53:36–39, 1995. **The sensitivity of one stool examination was compared with that of three examinations. The additional examinations increased the percent positive results as follows: 22.7% increase for *Entamoeba histolytica*, 11.3% increase for *Giardia lamblia*, and 31.1% increase for *Dientamoeba fragilis*. Even in symptomatic patients, these data indicate that examination of a single stool specimen will miss a large number of infections by pathogenic protozoa.**

[d] The ova and parasite examination and specific immunoassays should be separate, orderable, billable tests.

TABLE A1.20 Laboratory test reports: optional comments[a,b]

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1. ***Entamoeba histolytica/E. dispar* group**

   - Unless we see trophozoites containing ingested red blood cells (true pathogen: *E. histolytica*), we cannot tell from the organism morphology whether the patient has actual pathogenic *E. histolytica* organisms or nonpathogenic *E. dispar* present. Report as seen above: *Entamoeba histolytica/E. dispar* group (the word "group" is optional).

   Additional computer comments:

   - *Entamoeba* complex; unable to rule out the true pathogen, *E. histolytica*.
   - Unable to determine pathogenicity from organism morphology.
   - Unable to rule out true pathogen, *Entamoeba histolytica*.
   - Depending on patient's clinical condition, treatment may be appropriate.

   If you have the kit reagents to differentiate the two organisms,[c] comments could also be added:

   - If you wish to determine which of the two organisms is present, please submit a fresh stool specimen.
   - To determine the presence or absence of pathogenic *Entamoeba histolytica*, submit a fresh stool specimen.

2. **Identification of nonpathogens**

   Comments that can be used for reporting nonpathogens include the following. However, *these statements assume that a complete stool exam was performed on multiple stools*; you may detect nonpathogens in the first examination or an incomplete examination but miss a pathogen [example: *Dientamoeba fragilis* (pathogenic) requires the permanent stained smear for identification].

   Additional computer comments:

   - Considered nonpathogenic; treatment not recommended.
   - Nonpathogen; however, indication that patient has ingested something contaminated with fecal material.

3. **Reporting *Blastocystis* spp.**

   Several comments are optional for reporting *Blastocystis* spp.

   - Comprising approximately 20 subtypes/species, half of which are pathogenic, half of which are nonpathogenic.
   - Pathogenicity cannot be determined by morphology.

   You may want to add a second comment:

   - Other organisms capable of causing diarrhea should also be ruled out.

4. **Reporting *Plasmodium* spp.**[d]

   If no organisms are seen:

   - Submission of a single blood specimen will not rule out malaria; submit additional bloods every 4–6 h for 3 days if malaria remains a consideration.

   If *Plasmodium* spp. are detected but you are unable to identify to the species level, report as follows.

   - *Plasmodium* spp. seen, unable to rule out *P. falciparum* or *P. knowlesi*.

   If *Plasmodium* spp. are detected in a possible mixed infection:

   - *Plasmodium* spp. seen, unable to rule out *P. falciparum* or *P. knowlesi*.

   If *P. malariae* or *P. falciparum* is identified:

   - Unable to rule out *P. knowlesi*; confirm travel history to Malaysia or neighboring areas.

   Negative for parasites using automated hematology instruments:

   - Automated hematology instruments will not detect low malaria parasitemias seen in immunologically naive patients (travelers).

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[a] It is important to remember that educational information for your clients is critical to the success of your test-reporting formats. The information in the table should be shared with your clients prior to changing your actual reporting formats. Your physician group may have a preference regarding additional comments. Information updates or newsletters are appropriate for this purpose.

[b] All of the comments in the table are optional, and wording can be changed to fit your circumstances. However, it is recommended that you select specific comments and try not to use "free text," so that everyone reports test results in the same way each time.

[c] It is important to remember that current fecal immunoassay kits for the detection of the *Entamoeba histolytica/E. dispar* group or differentiation between the true pathogen (*E. histolytica*) and the nonpathogen (*E. dispar*) require fresh or frozen fecal specimens; although preserved specimens (generally formalin base or some of the single-vial fixatives, Universal Fixative/no formalin/no mercury/no PVA/TOTAL-FIX) can be used for the fecal immunoassays for *Giardia lamblia* or *Cryptosporidium* spp., they cannot be used for *Entamoeba* spp. testing.

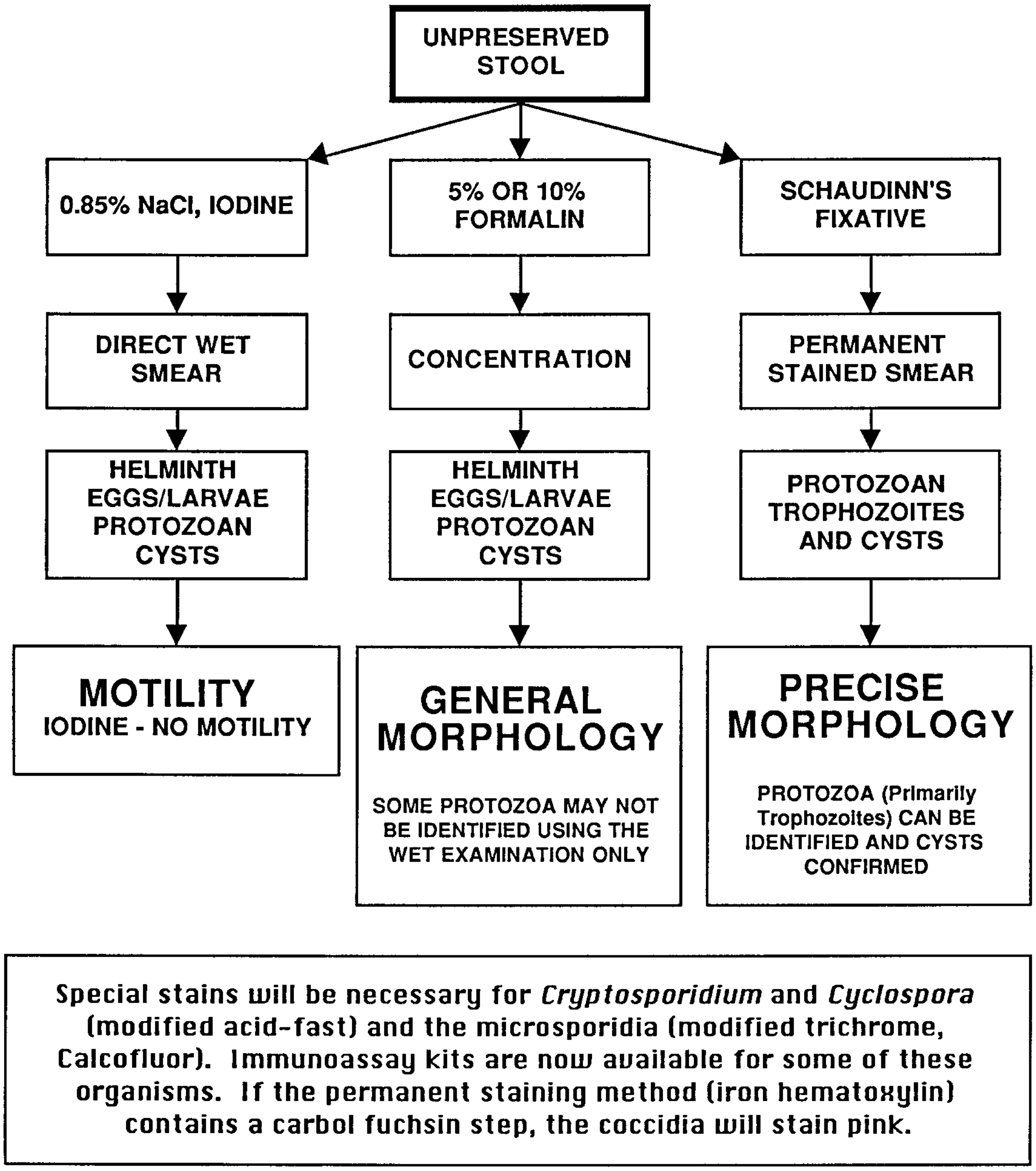
[d] **Note** Use of the BinaxNOW (Alere) malaria rapid test may help detection of mixed infections (STAT test). This test is FDA approved and the external malaria control is now also available (BinaxNOW Malaria Product Fact Sheet Test Kit & Positive Control). However, if test is negative, thick and thin blood film examination is mandatory (STAT).

TABLE A1.21 Estimated prevalence of parasitic diseases worldwide

Disease	Estimated prevalence figures
Protozoa	
Amebiasis	1% of population; annual deaths, 40,000–110,000
Giardiasis	200 million infected
Helminths (nematodes)	
Ascariasis	1.3 billion infected; annual deaths, 1,550; 320 million (35%) school-age children
Hookworm	1.3 billion infected; 233 million (25%) school-age children
Trichuriasis	900 million infected; 239 million (26%) school-age children
Strongyloidiasis	35 million infected
Trichostrongyliasis	5.5 million infected
Dracunculiasis	<100,000 infected
Helminths (cestodes)	65 million infected
Helminths (trematodes)	
Schistosomiasis	200 million infected; 500 million–600 million at risk
S. haematobium	100 million; 56 million (Africa only)
S. mansoni	60 million; 25 million (Africa only)
S. japonicum	1.5 million; annual deaths for all species, 500,000–1 million
Clonorchiasis and opisthorchiasis	13.5 million infected
Paragonimiasis	2.1 million infected
Fasciolopsiasis	10 million infected
Helminths (filiarial infections)	
Lymphatic filariasis	128 million infected; >40 million seriously incapacitated or disfigured; one-third of the people infected with the disease live in India, one-third are in Africa, and most of the remainder are in South Asia, the Pacific, and the Americas
Onchocerciasis	17.7 million infected
Blood parasites	
Malaria	400 million–490 million; annual deaths, 2.2 million–2.5 million; 40% of world's population at risk (3.2 billion); 853,000 deaths/yr in children <5 yr old (fifth leading cause of death in this age group)
Leishmaniasis	12 million infected; 1.5 million–2 million new cases each year; 90% are in 5 countries (Bangladesh, Brazil, India, Nepal, and Sudan); 90% of all cases of mucocutaneous leishmaniasis occur in Bolivia, Brazil, and Peru; 90% of all cases of cutaneous leishmaniasis occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia, and Syria, with 1 million–1.5 million new cases reported annually worldwide
African trypanosomiasis	300,000–500,000; 100,000 new cases each year; annual deaths, 5,000
American trypanosomiasis	24 million infected; 60,000 annual deaths

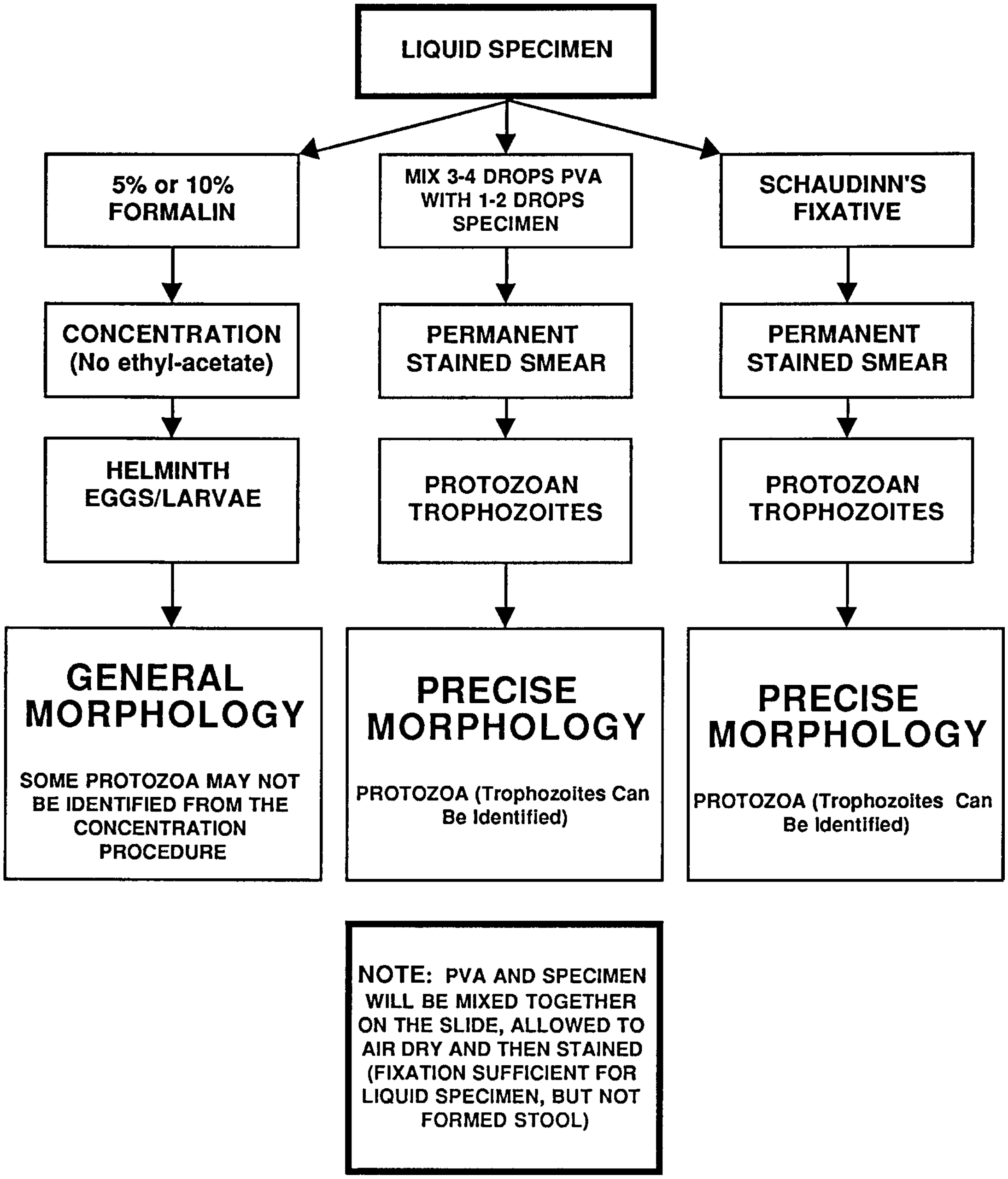
APPENDIX 2

Flowcharts and Staining Tables for Diagnostic Procedures

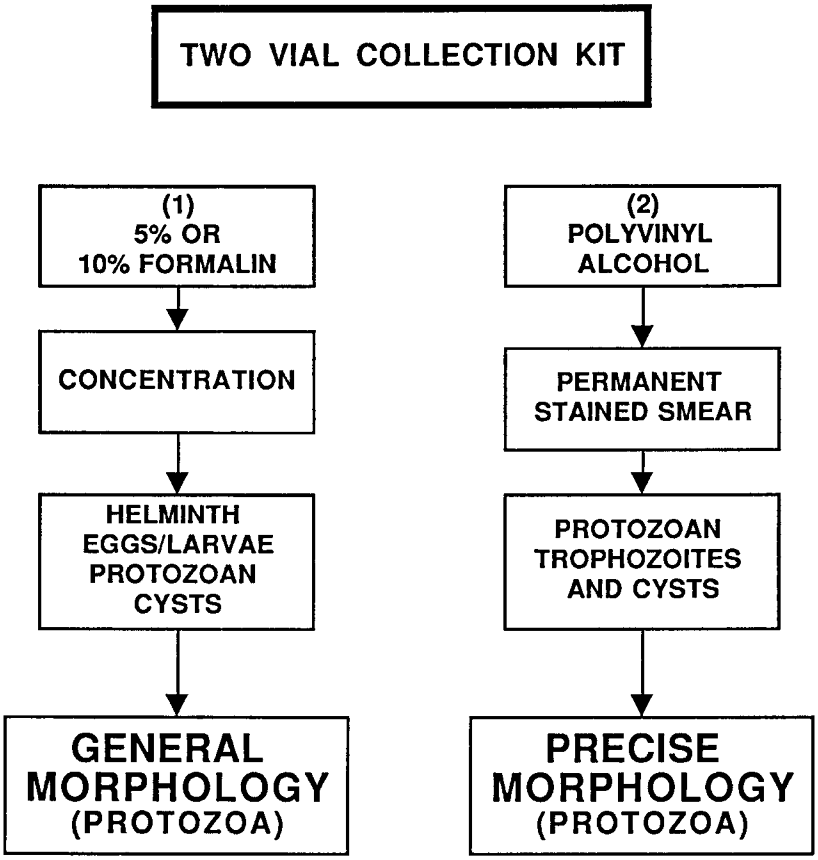

UNPRESERVED STOOL
0.85% NaCl, IODINE
5% OR 10% FORMALIN
SCHAUDINN'S FIXATIVE
DIRECT WET SMEAR
CONCENTRATION
PERMANENT STAINED SMEAR
HELMINTH EGGS/LARVAE PROTOZOAN CYSTS
HELMINTH EGGS/LARVAE PROTOZOAN CYSTS
PROTOZOAN TROPHOZOITES AND CYSTS
MOTILITY
IODINE - NO MOTILITY
GENERAL MORPHOLOGY
SOME PROTOZOA MAY NOT BE IDENTIFIED USING THE WET EXAMINATION ONLY
PRECISE MORPHOLOGY
PROTOZOA (Primarily Trophozoites) CAN BE IDENTIFIED AND CYSTS CONFIRMED

Special stains will be necessary for *Cryptosporidium* and *Cyclospora* (modified acid-fast) and the microsporidia (modified trichrome, Calcofluor). Immunoassay kits are now available for some of these organisms. If the permanent staining method (iron hematoxylin) contains a carbol fuchsin step, the coccidia will stain pink.

Flowchart A2.1 Procedure for processing fresh stool for the ova and parasite examination. Schaudinn's fixative can be prepared using the mercuric chloride base or the zinc sulfate base; the copper sulfate base does not produce organism morphologic quality equal to that seen with the mercuric chloride or zinc sulfate bases. The permanent stained smear can be stained using iron hematoxylin or trichrome stains. This approach includes the direct wet smear, concentration, and the permanent stained smear (complete ova and parasite examination for fresh specimen that is liquid or very soft).

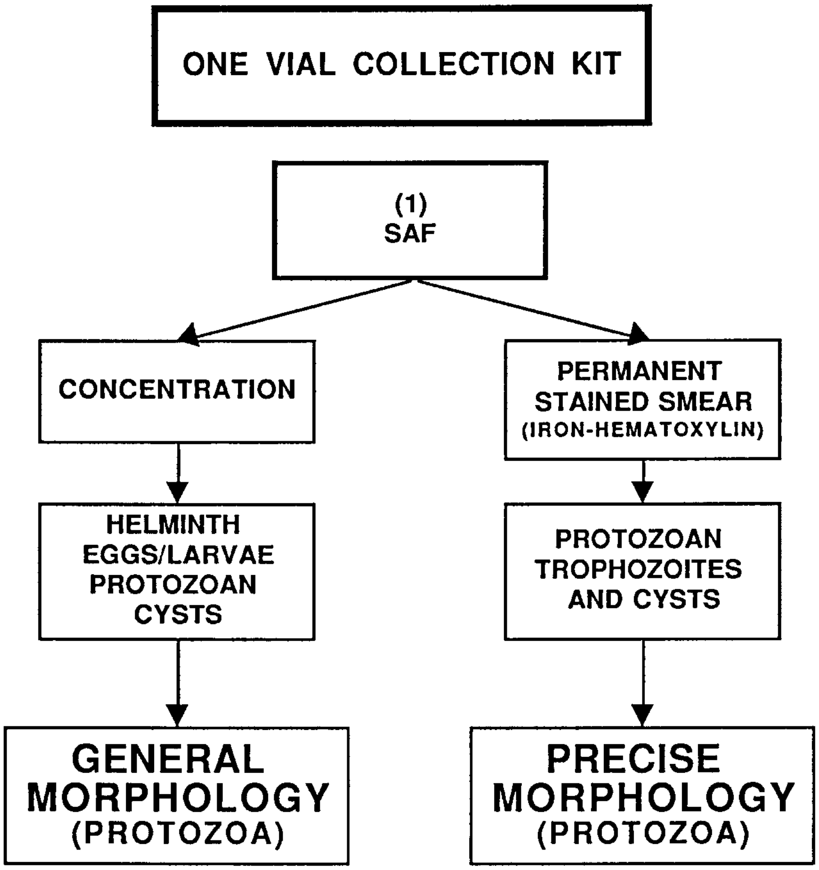

LIQUID SPECIMEN
5% or 10% FORMALIN
MIX 3-4 DROPS PVA WITH 1-2 DROPS SPECIMEN
SCHAUDINN'S FIXATIVE
CONCENTRATION (No ethyl-acetate)
PERMANENT STAINED SMEAR
PERMANENT STAINED SMEAR
HELMINTH EGGS/LARVAE
PROTOZOAN TROPHOZOITES
PROTOZOAN TROPHOZOITES
GENERAL MORPHOLOGY
SOME PROTOZOA MAY NOT BE IDENTIFIED FROM THE CONCENTRATION PROCEDURE
PRECISE MORPHOLOGY
PROTOZOA (Trophozoites Can Be Identified)
PRECISE MORPHOLOGY
PROTOZOA (Trophozoites Can Be Identified)
NOTE: PVA AND SPECIMEN WILL BE MIXED TOGETHER ON THE SLIDE, ALLOWED TO AIR DRY AND THEN STAINED (FIXATION SUFFICIENT FOR LIQUID SPECIMEN, BUT NOT FORMED STOOL)

Flowchart A2.2 Procedure for processing liquid specimens for the ova and parasite examination. If the volume of the liquid stool is quite small, mixing some of the specimen with polyvinyl alcohol (PVA) fixative (Schaudinn's fixative or other fixative containing PVA plastic powder that serves as the adhesive to "glue" the fecal material onto the glass slide) directly on the slide is recommended. Make sure that the slide is thoroughly dry before staining. NOTE: PVA FIXATIVE REFERS TO ANY LIQUID FIXATIVE INTO WHICH PVA PLASTIC POWDER HAS BEEN ADDED AS AN ADHESIVE TO "GLUE" THE FECAL MATERIAL ONTO THE SLIDE.

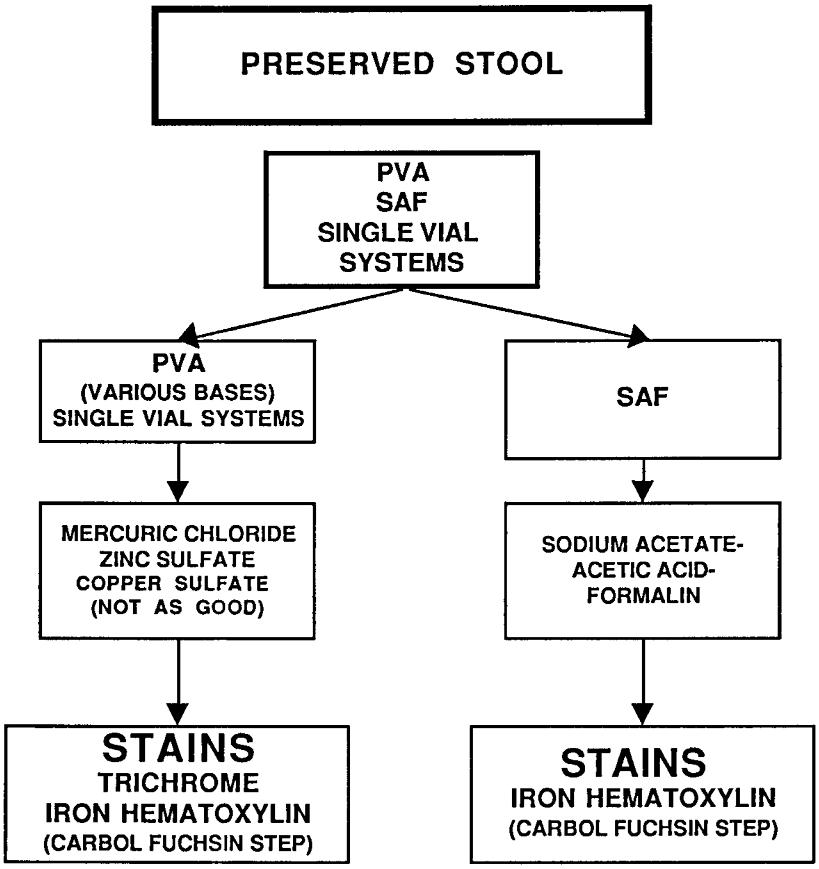

TWO VIAL COLLECTION KIT
(1)
5% OR
10% FORMALIN
CONCENTRATION
HELMINTH
EGGS/LARVAE
PROTOZOAN
CYSTS
GENERAL
MORPHOLOGY
(PROTOZOA)
(2)
POLYVINYL
ALCOHOL
PERMANENT
STAINED SMEAR
PROTOZOAN
TROPHOZOITES
AND CYSTS
PRECISE
MORPHOLOGY
(PROTOZOA)

1. **MERCURIC CHLORIDE BASE: BEST PVA**
   (TRICHROME, IRON HEMATOXYLIN)
2. **ZINC BASE: CURRENT BEST SUBSTITUTE**
   (TRICHROME, IRON HEMATOXYLIN PROBABLY OK)
3. **COPPER SULFATE BASE: FAIR SUBSTITUTE**
   (TRICHROME, IRON HEMATOXYLIN - BOTH FAIR-POOR)
4. **SAF: GOOD SUBSTITUTE FOR PVA FIXATIVE**
   (IRON HEMATOXYLIN BEST, TRICHROME OK)

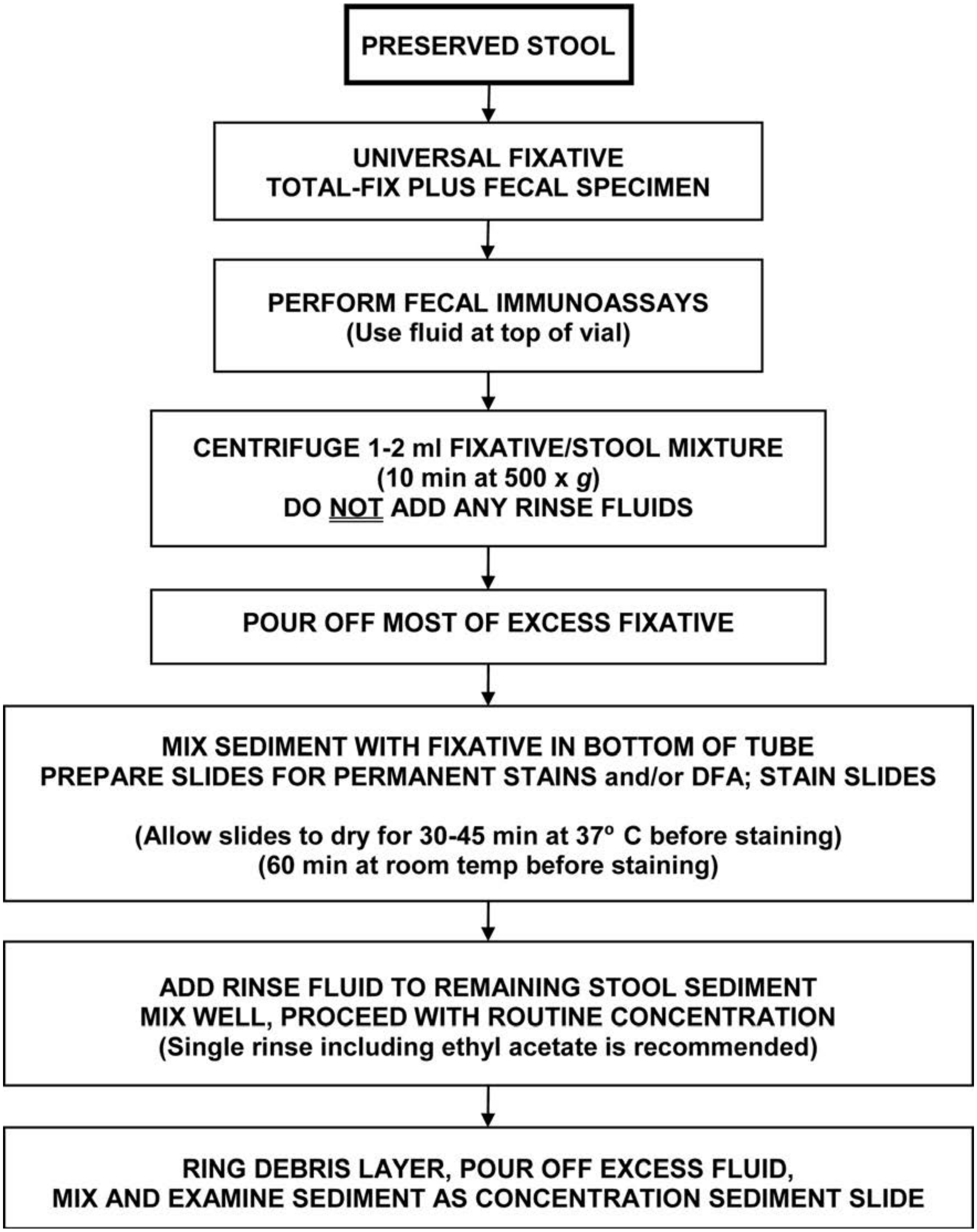
Flowchart A2.3 Procedure for processing preserved stool for the ova and parasite examination by using the traditional two-vial collection kit. This collection kit has been in use for many years. If the fecal specimen arrives in the laboratory in preservative, the direct wet mount for motility should not be performed. The complete ova and parasite examination consists of the concentration and permanent stained smear. PVA, polyvinyl alcohol; SAF, sodium acetate-acetic acid-formalin.


ONE VIAL COLLECTION KIT
(1) SAF
CONCENTRATION
PERMANENT STAINED SMEAR (IRON-HEMATOXYLIN)
HELMINTH EGGS/LARVAE PROTOZOAN CYSTS
PROTOZOAN TROPHOZOITES AND CYSTS
GENERAL MORPHOLOGY (PROTOZOA)
PRECISE MORPHOLOGY (PROTOZOA)

Flowchart A2.4 Procedure for processing sodium acetate-acetic acid-formalin (SAF)-preserved stool for the ova and parasite examination. SAF-preserved stool can be used with the fecal immunoassay kits (fluorescent-antibody assay, enzyme immunoassay, and cartridge formats for *Giardia lamblia* and *Cryptosporidium* spp.; kits for the true pathogen *Entamoeba histolytica* or the *Entamoeba histolytica/E. dispar* group require fresh or frozen stools); modified acid-fast stains for coccidia and modified trichrome stains can also be performed from SAF-preserved stool.


PRESERVED STOOL
PVA
SAF
SINGLE VIAL
SYSTEMS
PVA
(VARIOUS BASES)
SINGLE VIAL SYSTEMS
SAF
MERCURIC CHLORIDE
ZINC SULFATE
COPPER SULFATE
(NOT AS GOOD)
SODIUM ACETATE-
ACETIC ACID-
FORMALIN
STAINS
TRICHROME
IRON HEMATOXYLIN
(CARBOL FUCHSIN STEP)
STAINS
IRON HEMATOXYLIN
(CARBOL FUCHSIN STEP)

Flowchart A2.5 Use of various fixatives and their recommended stains. Fecal specimens preserved using polyvinyl alcohol (PVA) fixative (liquid fixative into which PVA plastic powder has been added) can be stained using either the iron hematoxylin or trichrome stains. However, fecal specimens preserved using sodium acetate-acetic acid-formalin appear to stain better using the iron hematoxylin approach. The single-vial preservatives (proprietary formulas) can be stained with iron hematoxylin, trichrome, or a manufacturer's stain prepared to accompany a particular fixative. *It is important to remember that parasite morphology (particularly intestinal protozoa) may not be as precise or clear as that seen when stool specimens are preserved in Schaudinn's fixative prepared with the mercuric chloride base (gold standard).*


PRESERVED STOOL
UNIVERSAL FIXATIVE
TOTAL-FIX PLUS FECAL SPECIMEN
PERFORM FECAL IMMUNOASSAYS
(Use fluid at top of vial)
CENTRIFUGE 1-2 ml FIXATIVE/STOOL MIXTURE
(10 min at 500 x g)
DO NOT ADD ANY RINSE FLUIDS
POUR OFF MOST OF EXCESS FIXATIVE
MIX SEDIMENT WITH FIXATIVE IN BOTTOM OF TUBE
PREPARE SLIDES FOR PERMANENT STAINS and/or DFA; STAIN SLIDES
(Allow slides to dry for 30-45 min at 37° C before staining)
(60 min at room temp before staining)
ADD RINSE FLUID TO REMAINING STOOL SEDIMENT
MIX WELL, PROCEED WITH ROUTINE CONCENTRATION
(Single rinse including ethyl acetate is recommended)
RING DEBRIS LAYER, POUR OFF EXCESS FLUID,
MIX AND EXAMINE SEDIMENT AS CONCENTRATION SEDIMENT SLIDE

Flowchart A2.6 Use of various fixatives and their recommended stains. Fecal specimens preserved in the Universal Fixative, TOTAL-FIX, can be stained using trichrome or iron hematoxylin. Using this processing algorithm, modified acid-fast stains (coccidia) and modified trichrome stains (microsporidia) can also be performed.

TABLE A2.1 Steps in the trichrome staining procedure (mercuric chloride-based PVA-preserved stool specimens)

Steps in the procedure	Time	Comments
Prepare slide for staining		Can be prepared from Schaudinn's liquid fixative or from mercuric chloride-based polyvinyl alcohol (PVA)
70% ethanol	5 min	Rinses off excess Schaudinn's fixative
70% ethanol + iodine	5 min	Color should look like strong tea; iodine will remove mercury from fecal smears; 10 min may be better if staining a full rack of slides[a]
70% ethanol	5 min	Rinses off excess iodine
70% ethanol	5 min	Completes removal of iodine
Trichrome stain[b]	10 min (variable)	Both mercury and iodine have been removed prior to placing the smears in stain
90% ethanol + acetic acid	Several dips	This begins the destaining process; it is important to move the smears rapidly from this dish to the next so that the destaining does not continue too long
95 or 100% ethanol	Several dips	This step removes the acidified alcohol, preventing the decolorizing step from continuing
100% ethanol	3–5 min	This step begins the dehydration process, in which all moisture is removed from the smear
100% ethanol	3–5 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process completed
Permount slides or allow them to air dry with no coverslip (see Table A2.7)		Permounted slides should be allowed to dry; they can be placed on trays and put in the incubator for 30 min or overnight; room temperature overnight is also sufficient

[a] The iodine-alcohol does not require an exact concentration. The color should not be too dark or the iodine will stain the protozoa, interfering with the subsequent trichrome staining; however, the color should not be too light or the mercuric chloride in the fixative will not be removed and mercuric chloride residue in the stained fecal smear will interfere with examination. Again, a strong-tea color is preferred (do not use "port wine" as a potential color intensity guide; this color is too dark).

[b] The trichrome staining procedure is a "regressive staining method" in which the fecal smears are overstained and then destained to provide better color and contrast.

TABLE A2.2 Steps in the trichrome staining procedure (non-mercuric chloride-based PVA-preserved stool specimens)

Steps in the procedure	Time	Comments
Prepare slide for staining		Can be prepared from non-mercuric chloride-based Schaudinn's fixative, with polyvinyl alcohol (PVA); examples would be Schaudinn's fixative prepared with the zinc sulfate or copper sulfate bases
70% ethanol	5 min	This dish is optional; a rinse can be used for dry smears (PVA) or from liquid Schaudinn's liquid fixative
Trichrome stain[a]	10 min (variable)	Since no mercury is present on the smear, there is no need to use the iodine dish; **however, when staining proficiency testing specimens that have been preserved in mercuric chloride-based fixatives, the iodine dish and subsequent rinse steps must be added back into your staining protocol; some proficiency testing agencies are now using fecal specimens preserved in fixatives containing no mercury; it is important to know what type of fixative was used prior to staining**
90% ethanol + acetic acid	Several dips	This begins the destaining process; it is important to move the smears rapidly from this dish to the next so that the destaining does not continue too long
95 or 100% ethanol	Several dips	This step removes the acidified alcohol, preventing the decolorizing step from continuing
100% ethanol	3–5 min	This step begins the dehydration process, in which all moisture is removed from the smear
100% ethanol	3–5 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process completed
Permount slides or allow them to air dry with no coverslip (see Table A2.7)		Permounted slides should be allowed to dry; they can be placed on trays and put in the incubator for 30 min or overnight; room temperature overnight is also sufficient

[a] The trichrome staining procedure is a "regressive staining method" in which the fecal smears are overstained and then destained to provide better color and contrast.

TABLE A2.3 Steps in the iron hematoxylin staining procedure (mercuric chloride-based PVA-preserved stool specimens) (Spencer-Monroe method)

Steps in the procedure	Time	Comments
Prepare slide for staining		Can be prepared from Schaudinn's liquid fixative or mercuric chloride-based polyvinyl alcohol (PVA); sodium acetate-acetic acid-formalin (SAF)-preserved specimens are also recommended
70% ethanol	5 min	Rinses off excess Schaudinn's fixative
70% ethanol + iodine	5 min	Color should look like strong tea; iodine will remove mercury from fecal smears; 10 min may be better if staining a full rack of slides[a]
70% ethanol (begin the process for SAF-preserved smears or non-mercuric chloride-preserved specimens at this point)	5 min	Rinses off excess iodine
Rinse in running tap water	10 min	Use a constant stream of water running into the container
Place in iron hematoxylin working solution	4–5 min	This step is the staining process
Rinse in running tap water	10 min	Use a constant stream of water running into the container
70% ethanol	5 min	This step begins the dehydration process in which all moisture is removed from the smear
95% ethanol	5 min	Dehydration process continues
100% ethanol	3–5 min	Dehydration process continues
100% ethanol	3–5 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process completed
Permount slides or allow them to air dry with no coverslip (see Table A2.7)		Permounted slides should be allowed to dry; they can be placed on trays and put in the incubator for 30 min or overnight; room temperature overnight is also sufficient

[a] The iodine-alcohol does not require an exact concentration. The color should not be too dark, or the iodine will stain the protozoa, interfering with the subsequent trichrome staining; however, the color should not be too light, or the mercuric chloride in the fixative will not be removed and mercuric chloride residue in the stained fecal smear will interfere with examination. Again, a strong-tea color is preferred (do not use "port wine" as a potential color intensity guide; this color is too dark).

TABLE A2.4 Steps in the iron hematoxylin staining procedure (mercuric chloride-based PVA-preserved stool specimens) (Tompkins-Miller method)

Steps in the procedure	Time	Comments
Prepare slide for staining		Can be prepared from Schaudinn's liquid fixative or mercuric chloride-based polyvinyl alcohol (PVA); sodium acetate-acetic acid-formalin (SAF)-preserved specimens are also recommended
70% ethanol	5 min	Rinses off excess Schaudinn's fixative
70% ethanol + iodine	5 min	Color should look like strong tea; iodine will remove mercury from fecal smears; 10 min may be better if staining a full rack of slides[a]
50% ethanol (begin the process for SAF-preserved smears or non-mercuric chloride-preserved specimens at this point)	5 min	Rinses off excess iodine
Rinse in running tap water	3 min	Use a constant stream of water running into the container
Place in 4% ferric ammonium sulfate mordant	5 min	This prepares the smear to take up the stain
Rinse in running tap water	1 min	Use a constant stream of water running into the container
Place in 0.5% aqueous hematoxylin	2 min	This begins the staining process
Rinse in running tap water	1 min	Use a constant stream of water running into the container
Place in 2% phosphotungstic acid	2–5 min	This provides the destaining process[b]
Rinse in running tap water	10 min	Use a constant stream of water running into the container
70% ethanol + a few drops of saturated aqueous lithium carbonate	3 min	The purpose of this step is to "blue up" the color
95% ethanol	5 min	This step begins the dehydration process in which all moisture is removed from the smear
100% ethanol	3–5 min	Dehydration process continues
100% ethanol	3–5 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process completed
Permount slides or allow them to air dry with no coverslip (see Table A2.7)		Permounted slides should be allowed to dry; they can be placed on trays and put in the incubator for 30 min or overnight; room temperature overnight is also sufficient

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[a] The iodine-alcohol does not require an exact concentration. The color should not be too dark or the iodine will stain the protozoa, interfering with the subsequent trichrome staining; however, the color should not be too light or the mercuric chloride in the fixative will not be removed and mercuric chloride residue in the stained fecal smear will interfere with examination. Again, a strong-tea color is preferred (do not use "port wine" as a potential color intensity guide; this color is too dark).

[b] Like the trichrome stain, this iron hematoxylin staining procedure is a "regressive staining method" in which the fecal smears are overstained and then destained to provide better color and contrast.

TABLE A2.5 Steps in the iron hematoxylin staining procedure (incorporating the carbol fuchsin step)[a]

Steps in the procedure	Time	Comments
Prepare slide for staining		Can be prepared from SAF-preserved specimens (SAF sediment placed on albumin-coated slides); **specimens preserved in Schaudinn's fixative and/or PVA are not recommended** (**any preservative containing mercuric chloride or PVA powder is not recommended**)
70% ethanol	5 min	Helps "glue" the material onto the slide
Rinse in container of tap water	2 min	Do not use running water
Place in Kinyoun's stain	5 min	Stains for modified acid-fast characteristics
Rinse in running tap water	1 min	Use a constant stream of water running into the container
Acid-alcohol decolorizer (0.5% acid-alcohol)	4 min	This must not be too strong or *Cyclospora cayetanensis* will decolorize too much[b]
Rinse in running tap water	1 min	Use a constant stream of water running into the container
Place in iron hematoxylin working solution	8 min	This begins the staining process
Rinse in container of tap water	1 min	Do not use running water
Place in picric acid solution	3–5 min	This step differentiates the hematoxylin stain
Rinse in running tap water	10 min	Use a constant stream of water running into the container
70% ethanol + some ammonia to bring pH to 8.0	3 min	The purpose of this step is to "blue up" the color
95% ethanol	5 min	This step begins the dehydration process
100% ethanol	3–5 min	Dehydration process continues
100% ethanol	3–5 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process completed
Permount slides or allow them to air dry with no coverslip (see Table A2.7)		Permounted slides should be allowed to dry; they can be placed on trays and put in the incubator for 30 min or overnight; room temperature overnight is also sufficient

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[a] This staining approach not only stains the intestinal protozoa (iron hematoxylin) but also stains the coccidia (*Cryptosporidium* spp., *Cyclospora cayetanensis*, and *Cystoisospora belli*), which are modified acid-fast positive.

[b] This iron hematoxylin staining procedure is a "regressive staining method" in which the fecal smears are overstained and then destained to provide better color and contrast.

TABLE A2.6 Steps in the trichrome staining procedure (Universal Fixative [no mercury, no formalin, no PVA])

Steps in the procedure	Time	Comments
Preserve stool in fixative	Fix 30 min	Ratio of 1/3 stool, 2/3 fixative, MIX WELL
Perform fecal immunoassays, if requested		Use the fluid at the top if the vial; if vial is shaken, allow to stand so particulate matter will settle out (10 min)
Centrifugation, 10 min @ 500 × *g*	10 min	Add approximately 1–2 ml stool/fixative mix to a tube or device for centrifugation; DO NOT ADD ANY RINSE FLUIDS
Smear preparation		Pour off most of supernatant fluid; mix sediment with remaining fixative; prepare fecal smears for permanent staining (trichrome), special stains for coccidia or microsporidia, or DFA immnoassay
Concentration*		**SEE LAST TWO STEPS BELOW**
Smear drying time	30–45 min at 37°C, 60 min at room temp	This step ensures the smears are thoroughly dry prior to staining
70% ethanol	5 min	This dish is optional; a rinse can be used for dry smears
Trichrome stain[a]	10 min (variable)	Since no mercury is present on the smear, there is no need to use the iodine dish; **however, when staining proficiency testing specimens that have been preserved in mercuric chloride-based fixatives, the iodine dish and subsequent rinse steps must be added back into your staining protocol; some proficiency testing agencies are now using fecal specimens preserved in fixatives containing no mercury; it is important to know what type of fixative was used prior to staining**
90% ethanol + acetic acid	Several dips	This begins the destaining process; it is important to move the smears rapidly from this dish to the next so that the destaining does not continue too long
100% ethanol	Several dips	This step removes the acidified alcohol, preventing the decolorizing step from continuing
100% ethanol	3–5 min	This step begins the dehydration process, in which all moisture is removed from the smear
Xylene or xylene substitute	5–10 min	Dehydration process continues
Xylene or xylene substitute	5–10 min	Dehydration process completed
Permount slides or allow them to air dry with no coverslip (see Table A2.7)		Permounted slides should be allowed to dry; they can be placed on trays and put in the incubator for 30 min or overnight; room temperature overnight is also sufficient
Concentration*	10 min	Add rinse fluid to remainder of stool sediment and proceed with the routine concentration (500 × *g* for 10 min) Single rinse plus ethyl acetate step is recommended
Prepare concentration for examination*	2 min	Ring debris layer, pour off excess fluid, mix, and examine sediment as concentration sediment slide

[a] The trichrome staining procedure is a "regressive staining method" in which the fecal smears are overstained and then destained to provide better color and contrast.

TABLE A2.7 Oil-mounted permanent stained smears (no Permount is used)

Steps in the procedure	Time	Comments
Remove stained smears from last dehydration step (xylene or xylene substitute) and place them flat on paper towels to dry	Allow to dry for 10 min	If using a xylene substitute, the smears will take longer to dry; leave on paper towels for 15–20 min
Once the fecal smears are dry, place 1 to 2 drops of immersion oil onto the dry fecal smear	3–5 min	Allow the oil to "sink into" the dry fecal smear; if the smears are somewhat thick, wait 10–15 min before microscopic examination
Place a no. 1 coverslip onto the fecal smear/oil combination	No waiting time required	A 22- by 22-mm coverslip is recommended; the addition of the coverslip will prevent damage to the oil lens when microscopic examination begins; when fecal material on the smear has been thoroughly dehydrated, the material may be hard/brittle and could scratch the oil lens if not protected
Add 1 drop of immersion oil to the top of the coverslip[a]	No waiting time required	The fecal smear is now ready to examine using the oil immersion objective (100× lens for a total magnification of ×1,000)

[a] The layers are as follows: glass slide, fecal smear, immersion oil, coverslip, immersion oil; this approach works quite well and is a substitute for the use of Permount or other mounting fluids. If a positive smear is found, the oil can be removed in xylene or xylene substitute and mounted with Permount and a coverslip in the normal way. The negative slides can be stored in a slide box that is recycled every month or so, and the old slides can then be discarded.

TABLE A2.8 Tips on stool processing and staining

Processing or staining step	Commentary and tips
Direct wet mount, water or saline	In general, organisms do not like distilled water; even in saline, trophozoites may not maintain adequate morphology very long; even with viable organisms, motility may be difficult to see (lower microscope light intensity); direct wet mounts should be prepared on liquid or very soft stools only (more formed stools usually do not contain any trophozoites)
Direct wet mount, iodine	If the iodine is too dark, it may interfere with helminth egg detection; eggs resemble debris and/or artifacts; internal protozoan morphology may be too dark; iodine should be the color of strong tea; iodine kills the organisms, so that no motility will be seen
Concentration-sedimentation	Rinse solutions can be water, saline, or formalin (regular 5% or 10% solution); do not rinse too many times (the supernatant fluid does *not* have to be clear); although a cleaner sediment may be easier to read, too much rinsing results in loss of organisms; a dirtier sediment is preferred to maximize organism detection; do not make coverslip preparations too thick (thinner is preferable); every centrifugation step should be at 500 × *g* for 10 min
Concentration-flotation	Rinse solutions can be water, saline, or formalin (regular 5% or 10% solution); do not rinse too many times (the supernatant fluid does *not* have to be clear); the more you rinse, the more likely you are to lose organisms each time; many labs use a single rinse or two at the most; it is important to use zinc sulfate of the correct specific gravity (1.18 for non-formalin-fixed material, 1.20 for specimens received in formalin); ***both*** **the surface film and sediment must be examined (not all parasites float in the zinc sulfate method)**
Permanent stained smear: smear preparation	The smear should be neither too thick nor too thin (you should be able to read newsprint through the fecal smear); if using polyvinyl alcohol (PVA)-preserved specimens, make sure that the excess PVA is removed from the stool prior to smear preparation (absorb excess PVA from specimen by placing a bit of specimen onto paper towels, wait a few minutes, and then prepare smears); smears should be thoroughly dry prior to staining (with the exception of using liquid Schaudinn's fixative); **formalin-fixed stool is not appropriate for permanent staining**
Permanent stained smear: staining	If specimen is preserved in a fixative containing mercuric chloride, it is mandatory that all mercury be removed prior to staining (iodine and subsequent alcohol steps); dehydration (moisture removal) must be complete or protozoan morphology will be difficult to see; the best dehydration is obtained with absolute ethanol; if the "commercial" absolute alcohol is used (ethanol plus other alcohols), the dehydration is not as complete, so dishes containing this solution must be changed more often (at least weekly and every few days if staining more than a few slides at a time); xylene substitutes do not dehydrate as well as xylene (dishes must be changed more often); xylene substitutes take longer to dry; however, slides must be thoroughly dry prior to mounting with immersion oil (see Table A2.7); **formalin-fixed stool is not appropriate for permanent staining**
Permanent stained smear: specimen preparation and staining (Universal Fixative)	See Appendix 2.6 for complete protocol using the Universal Fixative (incorporates guidelines for fecal immunoassays, concentration, routine permanent stained smear [e.g., trichrome], and special stains for coccidia [modified acid fast] and microsporidia [modified trichrome])

APPENDIX 3

Common Problems in Parasite Identification

The drawings (except Figure A3.27) are reprinted from Isenberg HD (ed), *Clinical Microbiology Procedures Handbook*, 2nd ed, ASM Press, Washington, DC, 2004. Other confusing artifacts can be seen in chapter 34.

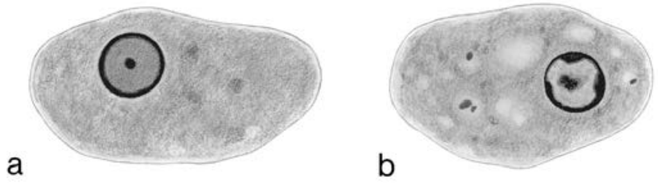


Figure A3.1 (a) *Entamoeba histolytica*/*E. dispar* trophozoite. Note the evenly arranged nuclear chromatin, central compact karyosome, and relatively clean cytoplasm. (b) *Entamoeba coli* trophozoite. Note the unevenly arranged nuclear chromatin, eccentric karyosome, and messy cytoplasm. These characteristics are very representative of the two organisms. However, in actual clinical specimens, there is great overlap between morphologic characteristics. When examining a patient specimen with a suspected mixed infection, one looks for two populations of organisms. When the majority of organisms seen appear to be from one genus/species, a rare one or two unusual organisms may just be outliers of the same genus/species that look a bit different. (Illustration by Sharon Belkin.)

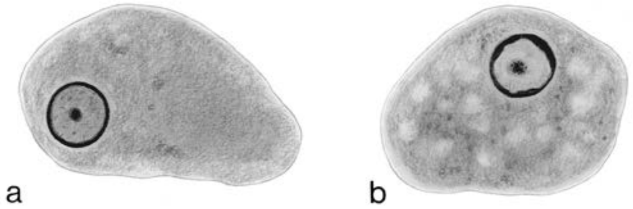


Figure A3.2 (a) *Entamoeba histolytica*/*E. dispar* trophozoite. Note the evenly arranged nuclear chromatin, central compact karyosome, and clean cytoplasm. (b) *Entamoeba coli* trophozoite. Note that the nuclear chromatin appears to be evenly arranged, the karyosome is central (but more diffuse), and the cytoplasm is messy, with numerous vacuoles and ingested debris. The nuclei of these two organisms tend to resemble one another (a very common finding in routine clinical specimens). However, the karyosome in *E. coli* tends to be larger and more blot-like. (Illustration by Sharon Belkin.)

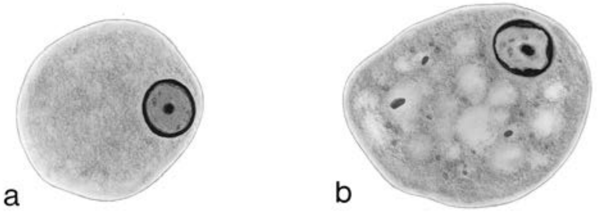


Figure A3.3 (a) *Entamoeba histolytica*/*E. dispar* trophozoite. Again, note the typical morphology (evenly arranged nuclear chromatin, central compact karyosome, and relatively clean cytoplasm). (b) *Entamoeba coli* trophozoite. Although the nuclear chromatin is eccentric, note that the karyosome seems to be compact and central. However, note the various vacuoles containing ingested debris. These organisms show some characteristics that are very similar (very common in clinical specimens). The nature of the karyosome tends to be very helpful; in *E. histolytica*/*E. dispar* the karyosome tends to be compact (may or may not be in the center). The karyosome of *E. coli* tends to be larger, more diffuse, and more irregular; it may or may not be eccentric. (Illustration by Sharon Belkin.)

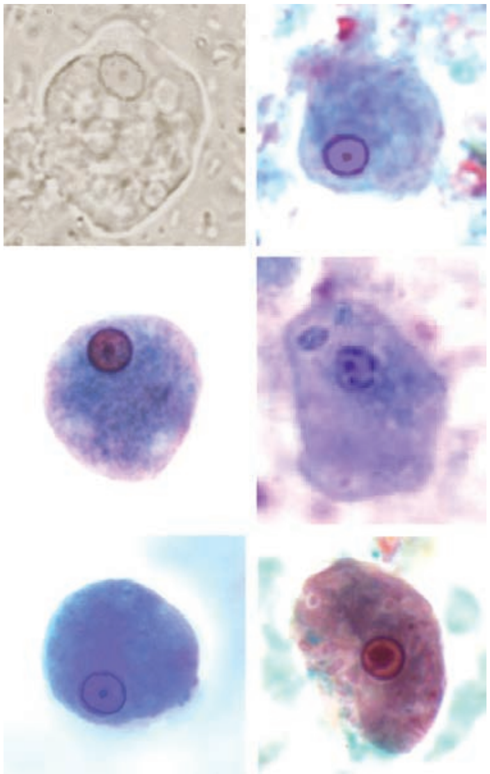


Figure A3.4 (Top) *Entamoeba histolytica*/*E. dispar* trophozoites shown in a wet preparation (left) and a permanent stained smear (right). (Middle) *E. histolytica*/*E. dispar* trophozoite in permanent stained smear (left); *Entamoeba coli* trophozoite in permanent stained smear (right). (Bottom) *E. histolytica*/*E. dispar* trophozoite in permanent stained smear (left); *E. coli* trophozoite in permanent stained smear (right). Note the differences in the nucleus and cytoplasm: *E. histolytica*/*E. dispar* has even nuclear chromatin, a central, compact karyosome, and clean cytoplasm, whereas *E. coli* has uneven nuclear chromatin, eccentric karyosome, and messy/dirty cytoplasm. However, often there are nuclear and cytoplasmic characteristics that are not specific for one species or another; too many characteristics are seen that resemble either or both species. Again, the important differences can be seen as two populations of organisms. It may be difficult to assign a species designation to every organism seen, while the overall set of characteristics generally allow the organisms to be assigned to one or more species. All images stained with Wheatley's trichrome stain (routine trichrome for fecal specimens).

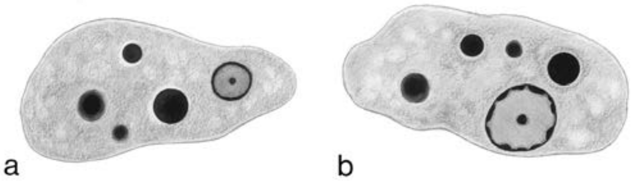
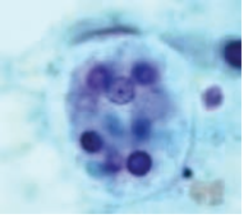
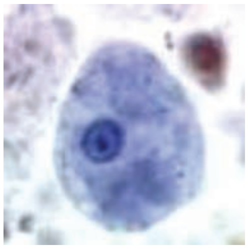
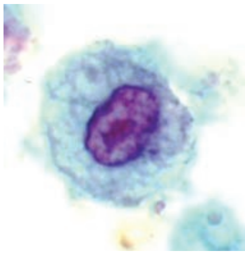


Figure A3.5 (a) *Entamoeba histolytica* trophozoite. Note the evenly arranged nuclear chromatin, central compact karyosome, and red blood cells (RBCs) in the cytoplasm. (b) Human macrophage. The key difference between the macrophage nucleus and that of *E. histolytica* is the size. Usually the ratio of nucleus to cytoplasm in a macrophage is approximately 1:6 or 1:8, while the true organism has a nucleus-to-cytoplasm ratio of approximately 1:10 or 1:12. The macrophage also contains ingested RBCs. In patients with diarrhea or dysentery, trophozoites of *E. histolytica* and macrophages are often confused, occasionally leading to a false-positive diagnosis of amebiasis when no parasites are present. Both the actual trophozoite and the macrophage may also be seen without ingested RBCs and can mimic one another. (Illustration by Sharon Belkin.)

TABLE A3.1 *Entamoeba* spp. trophozoites versus macrophages

Characteristic	***Entamoeba histolytica***	***E. dispar, E. moshkovskii, E. bangladeshi***	**Macrophage**
Size	12–25 µm	12–25 µm	Comparable to protozoa
Red blood cells (RBCs)	Round within vacuoles or may not be present	No RBCs	No RBCs
Nuclear/cytoplasmic ratio	1/10–1/12	1/10–1/12	1/6–1/8
			

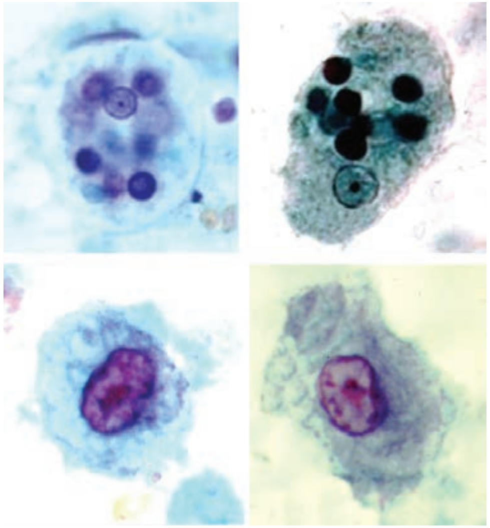


Figure A3.6 (Upper) *Entamoeba histolytica* trophozoites on permanent stained smears; note the evenly arranged nuclear chromatin, central, compact karyosome, and ingested RBCs. The presence of the ingested RBCs indicates the presence of the true pathogen, *E. histolytica*. Without the presence of ingested RBCs, the organism would have been identified as *E. histolytica*/*E. dispar* (it is impossible to determine pathogenicity from the morphology on the permanent stained smear). (Lower) Two macrophages from human fecal specimen. The main difference between the macrophage nucleus and that of *E. histolytica* is the size. Usually the ratio of nucleus to cytoplasm in a macrophage is approximately 1:6 or 1:8, while the true organism has a nucleus-to-cytoplasm ratio of approximately 1:10 or 1:12. All images but one stained with Wheatley's trichrome stain (routine trichrome for fecal specimens); upper right stained with iron-hematoxylin.

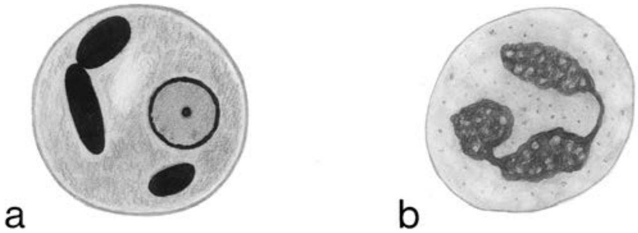
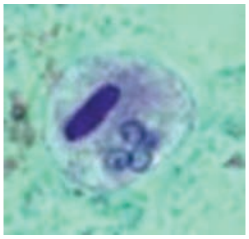
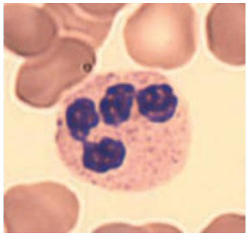
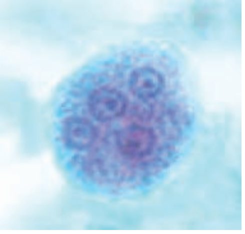
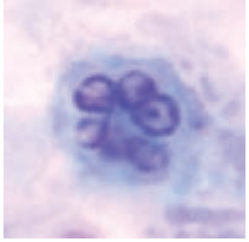


Figure A3.7 (a) *Entamoeba histolytica*/*E. dispar* precyst. Note the enlarged nucleus (prior to division) with evenly arranged nuclear chromatin and central compact karyosome. Chromatoidal bars (rounded ends with smooth edges) are also present in the cytoplasm. (b) Polymorphonuclear leukocyte (PMN). The nucleus is somewhat lobed (normal morphology) and represents a PMN that has not been in the gut very long. Occasionally, the positioning of the chromatoidal bars and the lobed nucleus of the PMN will mimic one another. The chromatoidal bars stain more intensely, but the shapes can overlap, as seen here. (Illustration by Sharon Belkin.)

TABLE A3.2 *Entamoeba* spp. cysts versus polymorphonuclear leukocytes (PMNs)

Characteristic	*E. histolytica, E. dispar, E. moshkovskii, E. bangladeshi*	PMNs
Size	10–15 µm	10–15 µm
RBCs	Not present	Not present
No. of nuclei	4	4–5, may be lobed (2–5 nuclear lobes)
Shape of nuclei	Usually round and consistent shape	Irregular shapes and may vary tremendously
		
		

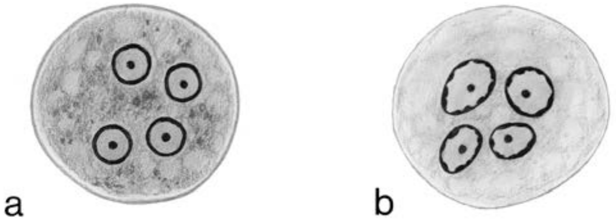
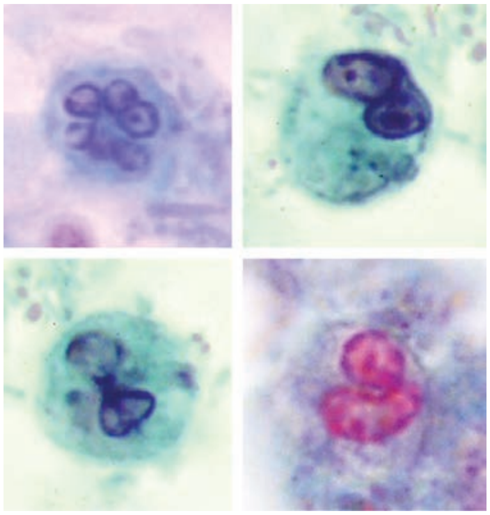


Figure A3.8 (a) *Entamoeba histolytica/E. dispar* mature cyst. Note that the four nuclei are very consistent in size and shape. (b) PMN. Note that the normal lobed nucleus has now broken into four fragments, which mimic four nuclei with peripheral chromatin and central karyosomes. When PMNs have been in the gut for some time and have begun to disintegrate, the nuclear morphology can mimic that seen in an *E. histolytica/E. dispar* cyst. However, human cells are often seen in the stool in patients with diarrhea; with rapid passage of the gastrointestinal tract contents, there will not be time for amebic cysts to form. Therefore, for patients with diarrhea and/or dysentery, if "organisms" that resemble the cell in panel b are seen, think first of PMNs, not *E. histolytica/E. dispar* cysts. (Illustration by Sharon Belkin.)

Figure A3.9 (Upper) PMNs. Note the appearance of "multiple nuclei" in the cell on the left, while the cell on the right contains the lobed nucleus, which has not yet fragmented to mimic an *Entamoeba* cyst. (Lower) PMNs in which the lobed nuclei have not yet fragmented; these cells should not be confused with *Entamoeba* cysts. When PMNs have been in the gut for some time and have begun to disintegrate, the nuclear morphology can mimic that seen in an *Entamoeba* cyst. However, remember that in patients with diarrhea, the gut contents move too rapidly for cysts to form; thus, cells with what appear to be multiple nuclei are actually PMNs.



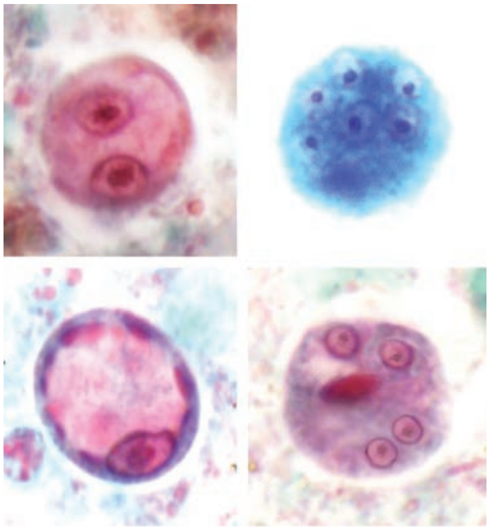
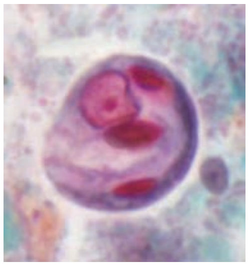
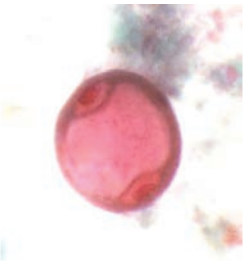
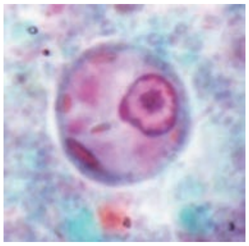
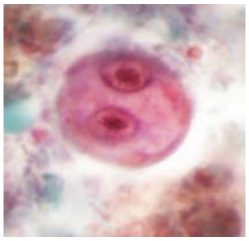
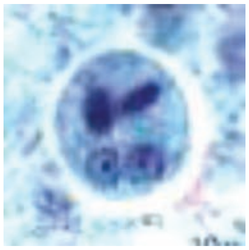
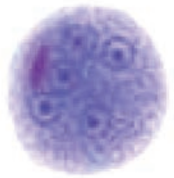
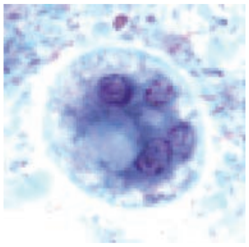
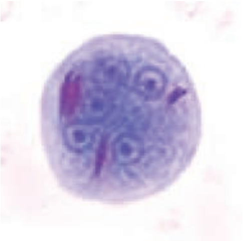


Figure A3.10 (Upper) *Entamoeba coli* precyst with two enlarged nuclei, one on each side of the precyst) (left) and *E. coli* cyst (five or more nuclei) (right). These cells could be confused with PMNs. (Lower) *Entamoeba histolytica/E. dispar* precyst on the left with a single enlarged nucleus; *E. histolytica/E. dispar* mature cyst (containing four nuclei and one chromatoidal bar) on the right. All images stained with Wheatley's trichrome stain (routine trichrome for fecal specimens).

TABLE A3.3 *Entamoeba histolytica* versus *Entamoeba coli* precysts and cysts

Characteristic	*E. histolytica, E. dispar, E. moshkovskii, E. bangladeshi*	*E. coli*
Size	10–15 μm	10–15 μm
RBCs	Not present	Not present
Precyst, no. of nuclei	1	2, one at each side
Precyst, shape of nuclei	Usually round	Often elongated
Precysts		
Precysts		
Cyst, no. of nuclei	4; some may not be in plane of focus	5 or more; some may not be in plane of focus
Cysts: chromatoidal bars	Chromatoidal bars have smooth, rounded ends	Chromatoidal bars have sharp, spiked edges
		
Cysts: chromatoidal bars may not always be visible		

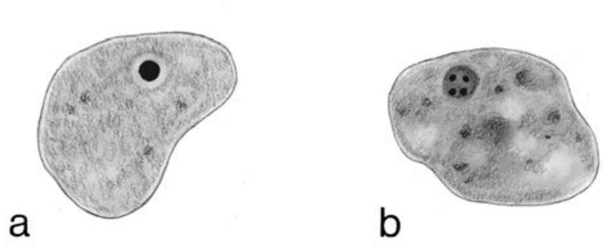


Figure A3.11 (a) *Endolimax nana* trophozoite. This organism is characterized by a large karyosome with no peripheral chromatin, although many nuclear variations are normally seen in any positive specimen. (b) *Dientamoeba fragilis* trophozoite. Normally, the nuclear chromatin is fragmented into several dots (often a "tetrad" arrangement). The cytoplasm is normally more "junky" than that seen in *E. nana*. If the morphology is typical, as in these two illustrations, differentiating between these two organisms is not very difficult. However, the morphologies of the two are often very similar. (Illustration by Sharon Belkin.)

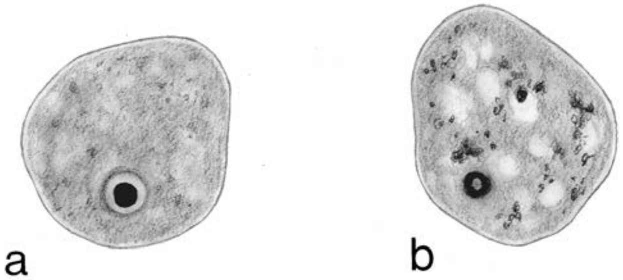
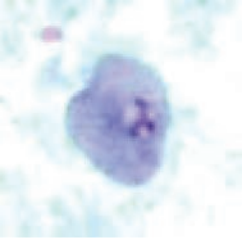
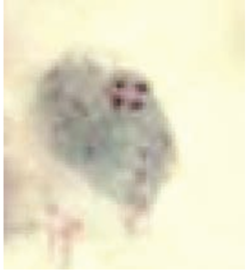
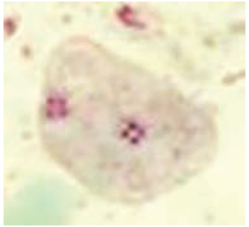
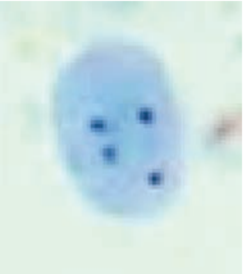
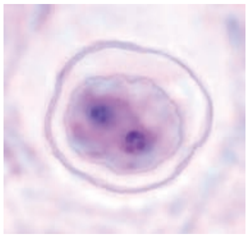


Figure A3.12 (a) *Endolimax nana* trophozoite. Note that the karyosome is large and surrounded by a "halo," with very little chromatin on the nuclear membrane. (b) *Dientamoeba fragilis* trophozoite. In this organism, the karyosome is beginning to fragment and there is a slight clearing in the center of the nuclear chromatin. If the nuclear chromatin has not become fragmented, *D. fragilis* trophozoites can very easily mimic *E. nana* trophozoites. This could lead to a report indicating that no pathogens were present when, in fact, *D. fragilis* is now considered a definite cause of symptoms. (Illustration by Sharon Belkin.)

TABLE A3.4 *Endolimax nana* versus *Dientamoeba fragilis*

Characteristic	***Endolimax nana***	***Dientamoeba fragilis***	**Comments**
Size, trophozoite	6–12 µm	5–15 µm	Both have wide size and shape range
No. of nuclei	1; tremendous nuclear variation in trophozoites	1 or 2; chromatin often fragmented; 1 nucleus	Large overlap in morphology; 2 nuclei
Trophozoites			
Size, cyst	5–10 µm	5–10 µm	
Cysts			If a single nucleus only, may be very difficult to differentiate the two organisms; *D. fragilis* is as common as *Giardia*.

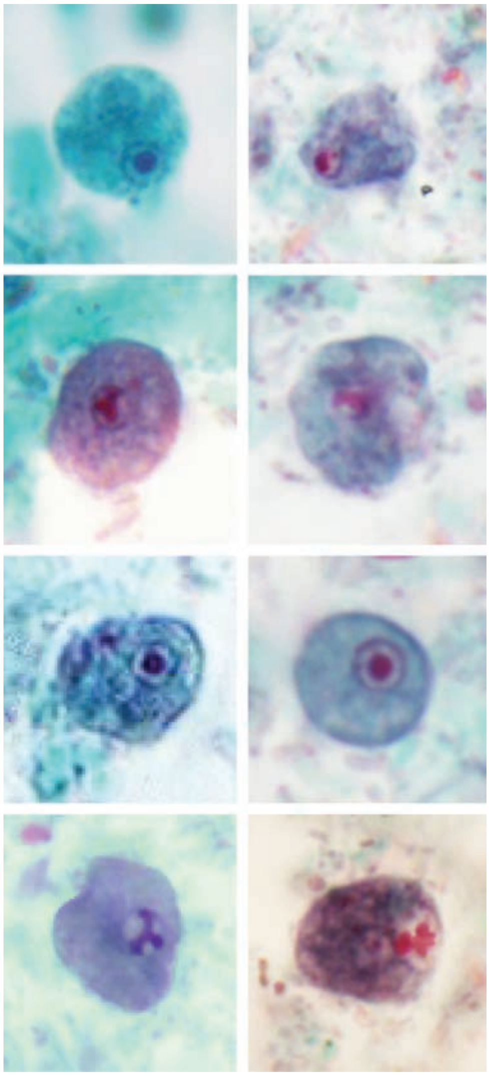


Figure A3.13 *Endolimax nana* trophozoites. Note the tremendous nuclear variation; there is more nuclear variation in this organism than any of the other intestinal protozoa. The karyosome appears quite different from organism to organism; they can mimic *Dientamoeba fragilis* and/or *Entamoeba hartmanni* (particularly if the nucleus appears to have peripheral chromatin like the organisms in row 3 from the top).

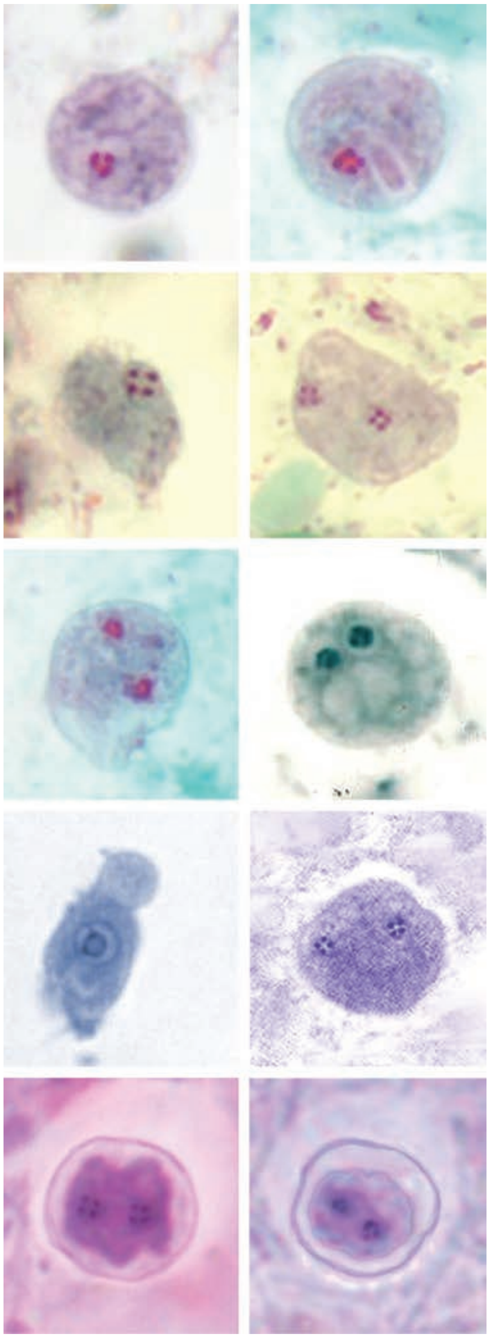


Figure A3.14 *Dientamoeba fragilis* trophozoites. (Top three rows) Some have a single nucleus, while others have two nuclei; the nuclei tend to fragment into several chromatin dots. (Row 4) The image on the left shows the clearing within the karyosome prior to fragmentation into chromatin dots. Both images in this row are stained using iron-hematoxylin. All other images are stained using the Wheatley's routine trichrome stain. (Bottom) These two images show the newly discovered cyst stage, with the double cyst wall (Stark D et al, *J Clin Microbiol* **52**:2680, 2014). However, the cystic forms tend to be rare in human specimens, a probable factor in the original failure to report and confirm these forms.

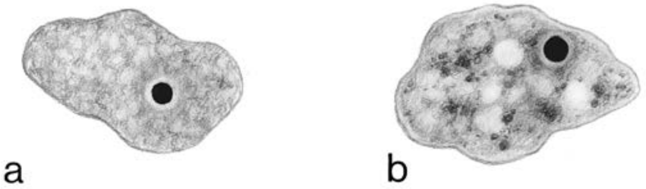


Figure A3.15 (a) *Endolimax nana* trophozoite. Note the large karyosome surrounded by a clear space. The cytoplasm is relatively clean. (b) *Iodamoeba bütschlii* trophozoite. Although the karyosome is similar to that of *E. nana*, note that the cytoplasm in *I. bütschlii* is much more heavily vacuolated and contains ingested debris. Often, these two trophozoites cannot be differentiated. However, the differences in the cytoplasm are often helpful. There is a definite size overlap between the two genera. (Illustration by Sharon Belkin.)

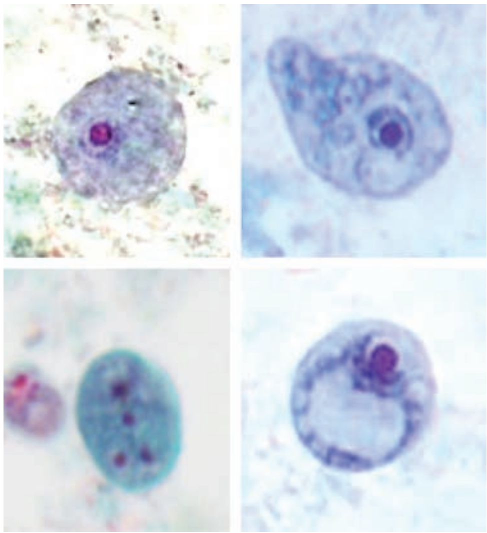


Figure A3.16 (Upper left) *Endolimax nana* trophozoite (note the large single karyosome with very little peripheral chromatin); (right) *Iodamoeba bütschlii* trophozoite (note the large karyosome, some light peripheral nuclear chromatin, and the messy/dirty cytoplasm containing many vacuoles). (Lower left) *E. nana* cyst (note the four karyosomes with no peripheral chromatin; shape tends to be somewhat round to oval); (right) *I. bütschlii* cyst (note the large vacuole, single nucleus with large karyosome, and chromatin granules at one edge of the nucleus [basket nucleus]).

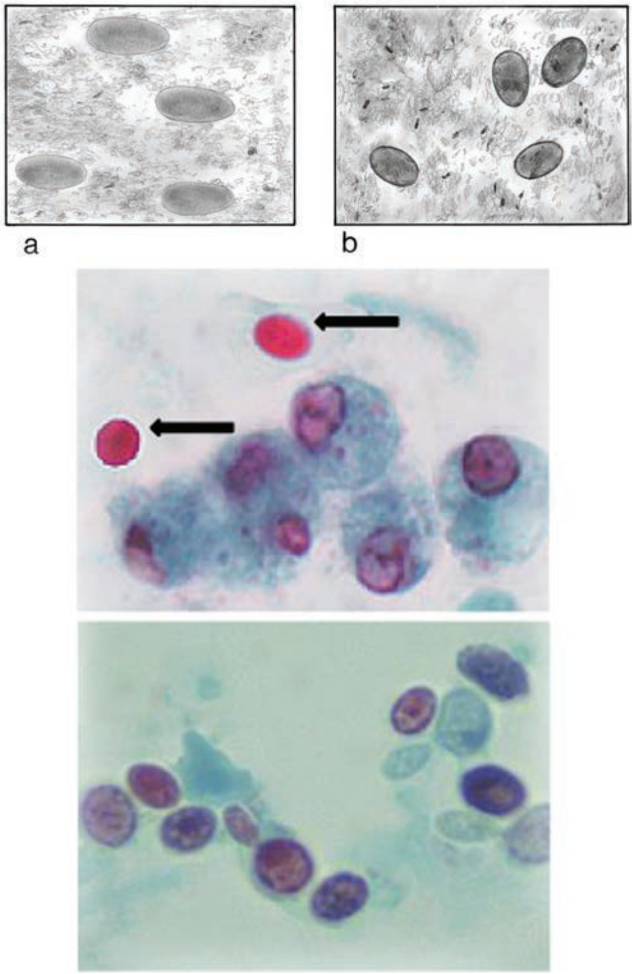


Figure A3.17 (Top) (a) RBCs on a stained fecal smear. Note that the cells are very pleomorphic but tend to be positioned in the direction in which the stool was spread onto the slide. (b) Yeast cells on a stained fecal smear. These cells tend to remain oval and are not aligned in any particular way on the smear. These differences are important when the differential identification is between *Entamoeba histolytica* containing RBCs and *Entamoeba coli* containing ingested yeast cells. If RBCs or yeast cells are identified in the cytoplasm of an organism, they must also be visible in the background of the stained fecal smear. (Illustration by Sharon Belkin.) (Middle) PMNs and RBCs (see arrows). The RBC cell shape can vary tremendously. (Bottom) Yeast cells in fecal material. Note the shape tends to be consistent and there appears to be no particular direction of placement such as is seen frequently with RBCs.

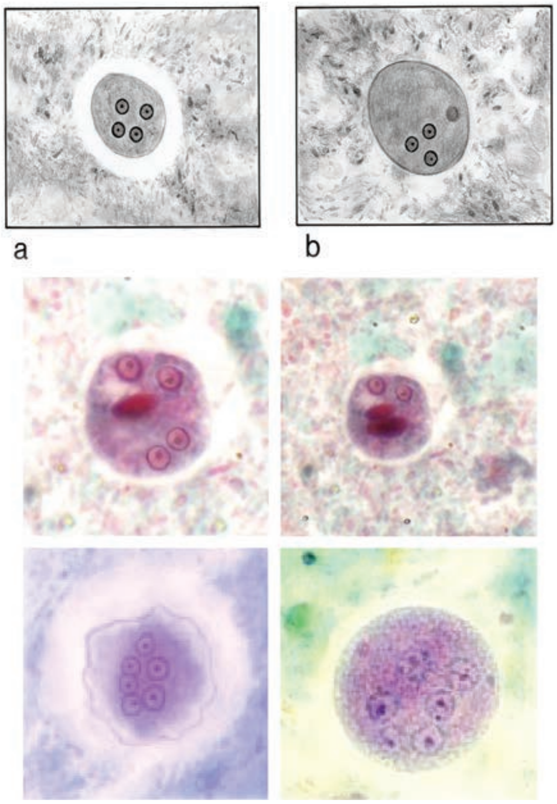


Figure A3.18 (Top) (a) *Entamoeba histolytica/E. dispar* cyst. Note the shrinkage due to dehydrating agents in the staining process. (b) *E. histolytica/E. dispar* cyst. In this case, the cyst exhibits no shrinkage. Only three of the four nuclei are in focus. Normally, this type of shrinkage is seen with protozoan cysts and is particularly important when a species is measured and identified as either *E. histolytica/E. dispar* or *Entamoeba hartmanni*. The whole area, including the halo, must be measured prior to species identification. If just the cyst is measured, the organism would be identified as *E. hartmanni* (nonpathogenic) rather than *E. histolytica/E. dispar* (possibly pathogenic). (Illustration by Sharon Belkin.) (Middle, left) *E. histolytica/E. dispar* cyst with four nuclei and one chromatoidal bar (note the shrinkage around the cyst); (right) *Entamoeba hartmanni* cyst with two of the four nuclei visible and two chromatoidal bars. (Note also the shrinkage around the cyst wall; this entire area would need to be measured for accurate species identification.) (Bottom) *Entamoeba coli* cysts. Note the shrinkage around the cyst wall; however, the identification to species could be made on the basis of five or more visible nuclei.

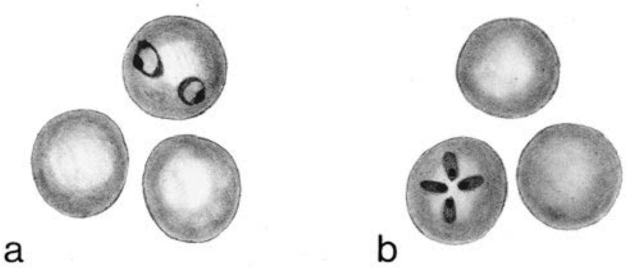


Figure A3.19 (a) *Plasmodium falciparum* rings. Note the two rings in the RBC. Multiple rings per cell are more typical of *P. falciparum* than of the other species causing human malaria. (b) *Babesia* rings. One of the RBCs contains four small *Babesia* rings. This particular arrangement is called the Maltese cross and is diagnostic for *Babesia* spp. (although this configuration is not always seen). *Babesia* infections can be confused with cases of *P. falciparum* malaria, primarily because multiple rings can be seen in the RBCs. Another difference involves ring morphology. *Babesia* rings are often of various sizes and tend to be very pleomorphic, while those of *P. falciparum* tend to be more consistent in size and shape. (Illustration by Sharon Belkin.)

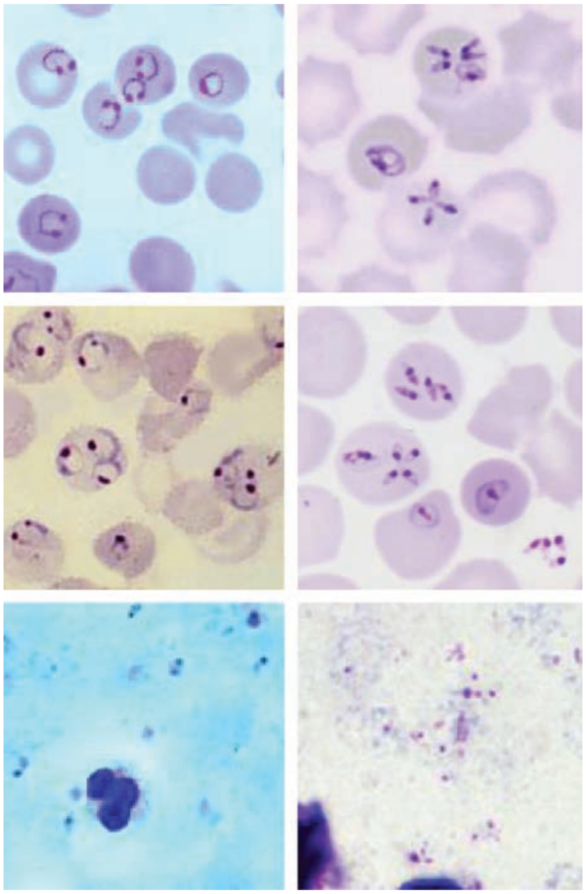


Figure A3.20 (Top, left) *Plasmodium falciparum* rings. Note the "clean" morphology and headphone appearance of some of the rings; (right) *Babesia* spp. Note the "messy/pleomorphic" rings and the presence of the Maltese cross configuration of four rings within a single RBC; this does not occur in every species of *Babesia*. (Middle, left) *P. falciparum* ring forms in a heavy infection (note the clearly defined rings); (right) *Babesia* spp.; note smaller rings. Also note the rings outside of the RBCs, which does not occur in malarial infections. (Bottom, left) *P. falciparum* rings in a thick film (note the nuclei and cytoplasm portion of the ring forms); (right) *Babesia* spp. thick film; note the nuclei are seen and the nuclei appear smaller than those seen in the malaria thick film. However, differentiation of organisms based on thick film morphology can be quite difficult. Identification is best achieved from the thin blood film.

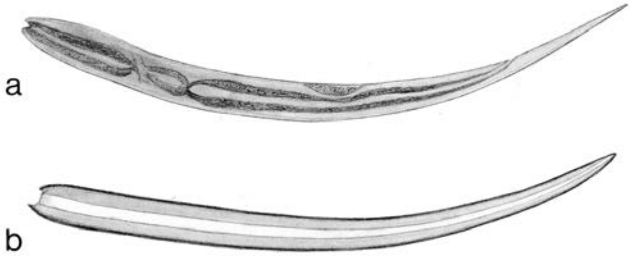


Figure A3.21 (a) *Strongyloides stercoralis* rhabditiform larva. Note the short buccal capsule (mouth opening) and the internal structure, including the genital primordial packet of cells. (b) Root hair (plant material). Note that there is no specific internal structure and the end is ragged where it was broken off from the main plant. Plant material often mimics some of the human parasites. This comparison is one of the best examples. These artifacts are occasionally submitted as proficiency-testing specimens. (Illustration by Sharon Belkin.)

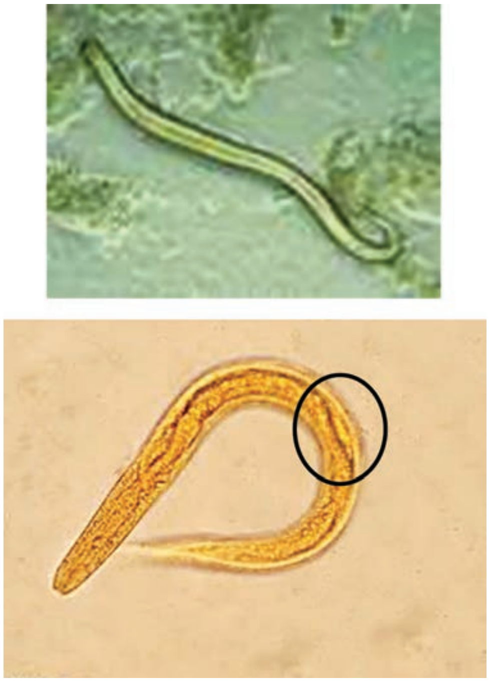


Figure A3.22 (Upper) Root hair artifact that resembles actual nematode larva. (Lower) *Strongyloides stercoralis* rhabditiform larva. Note the internal structures, especially the large, genital primordium packet of cells (circle).

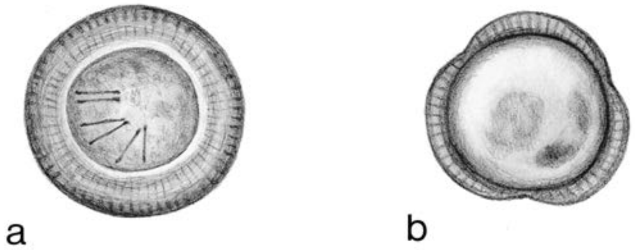


Figure A3.23 (a) *Taenia* egg. This egg has been described as having a thick, radially striated shell containing a six-hooked embryo (oncosphere). (b) Pollen grain. Note that this trilobed pollen grain has a similar type of "shell" and, if turned the right way, could resemble a *Taenia* egg. This similarity represents another source of confusion between a helminth egg and a plant material artifact. When examining fecal specimens in a wet preparation, you can tap on the coverslip to get objects to move around. As they move, you can see more morphologic detail. (Illustration by Sharon Belkin.)

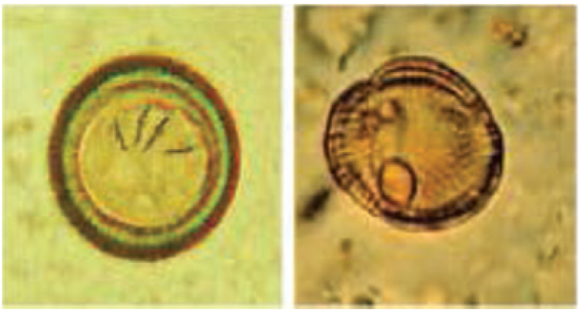


Figure A3.24 (Left) *Taenia* egg, showing the striated shell and six-hooked embryo (oncosphere) within the egg shell. (Right) Pollen grain that can resemble an actual *Taenia* egg. Note that the shape may vary, especially depending on the way the pollen grain is lying in the wet preparation.

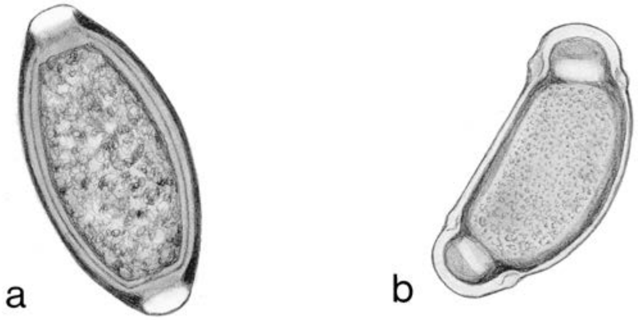


Figure A3.25 (a) *Trichuris trichiura* egg. This egg is typical and is characterized by the barrel shape with thick shell and two polar plugs. (b) Bee pollen. This artifact certainly mimics the *T. trichiura* egg. However, note that the shape is somewhat distorted. This is an excellent example of a parasite look-alike that could be confusing. (Illustration by Sharon Belkin.)

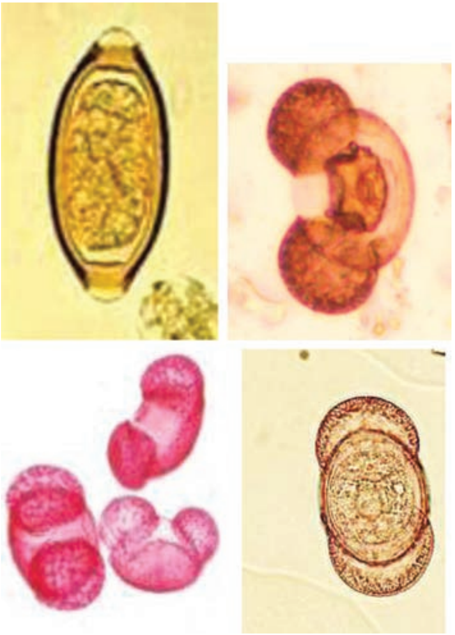


Figure A3.26 (Upper, left) *Trichuris trichiura* egg; (right) pollen that can mimic a *T. trichiura* egg. (Lower) Examples of pine pollen.

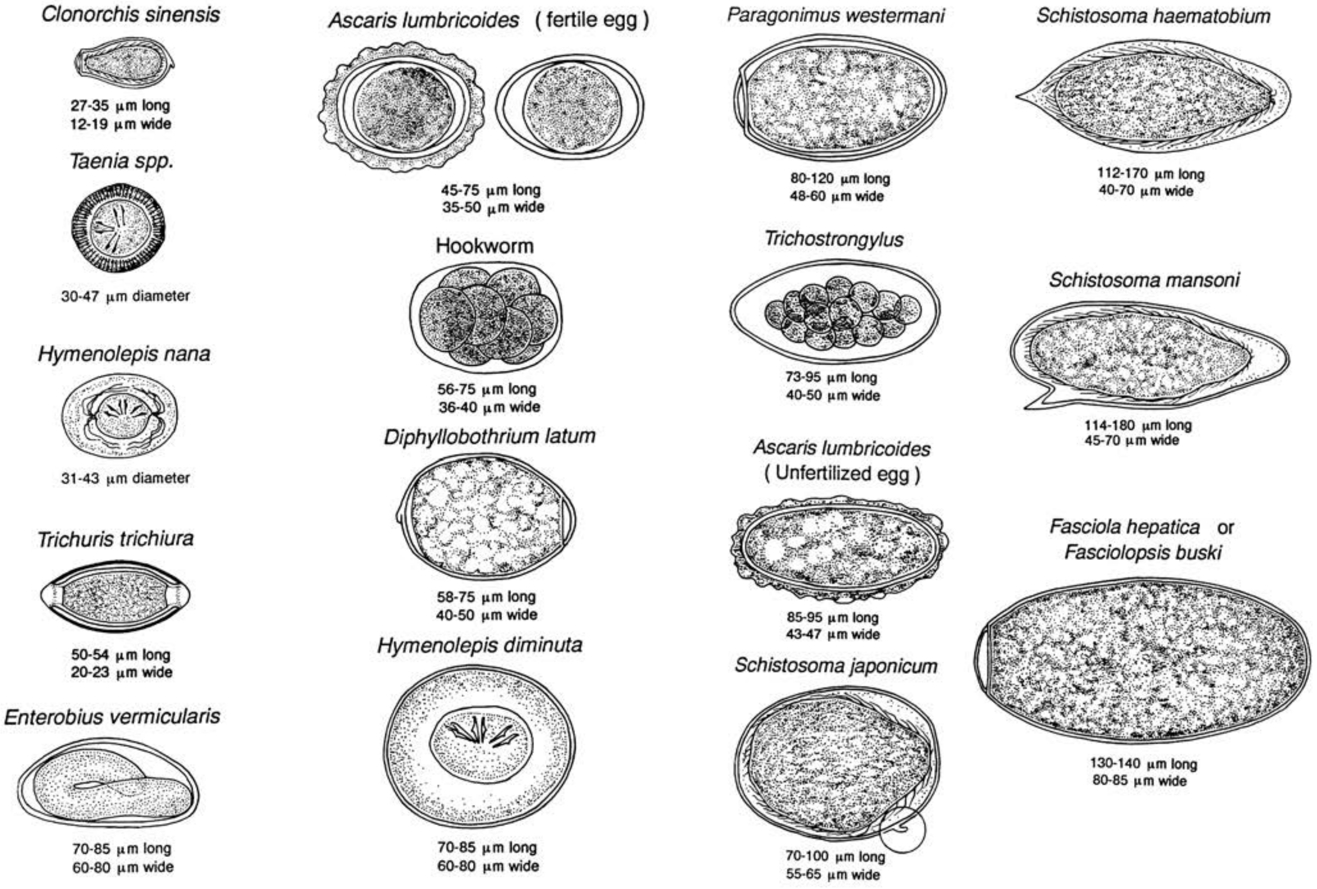

Clonorchis sinensis
27-35 µm long
12-19 µm wide
Taenia spp.
30-47 µm diameter
Hymenolepis nana
31-43 µm diameter
Trichuris trichiura
50-54 µm long
20-23 µm wide
Enterobius vermicularis
70-85 µm long
60-80 µm wide
Ascaris lumbricoides (fertile egg)
45-75 µm long
35-50 µm wide
Hookworm
56-75 µm long
36-40 µm wide
Diphyllobothrium latum
58-75 µm long
40-50 µm wide
Hymenolepis diminuta
70-85 µm long
60-80 µm wide
Paragonimus westermani
80-120 µm long
48-60 µm wide
Trichostrongylus
73-95 µm long
40-50 µm wide
Ascaris lumbricoides
(Unfertilized egg)
85-95 µm long
43-47 µm wide
Schistosoma japonicum
70-100 µm long
55-65 µm wide
Schistosoma haematobium
112-170 µm long
40-70 µm wide
Schistosoma mansoni
114-180 µm long
45-70 µm wide
Fasciola hepatica or
Fasciolopsis buski
130-140 µm long
80-85 µm wide

Figure A3.27 Relative sizes of helminth eggs.

TABLE A3.5 Adult nematodes and/or larvae found in stool specimens: size comparisons

Nematodes	Size
Ascaris lumbricoides	
Adult female	200–350 by 4–6 mm
Adult male	150–200 by 2–4 mm
Trichuris trichiura	
Adult female	35–50 mm
Adult male	30–45 mm
Enterobius vermicularis	
Adult female	8–13 mm
Adult male	2–5 mm
Hookworms (*Ancylostoma duodenale* and *Necator americanus*)	
Adult female	8–11 by 0.45 mm
Adult male	10–13 by 0.6 mm
Filariform larva	700 by 20 μm
Rhabditiform larva	250 by 20 μm
Strongyloides stercoralis	
Filariform larva	600 by 20 μm
Rhabditiform larva	250 by 20 μm
Trichinella spiralis	
Adult female	3–4 by 0.6 mm
Adult male	1.5 by 0.045 mm
Larva	90–100 by 6 μm
Microfilariae	
Wuchereria bancrofti	230–320 by 10 μm
Brugia malayi	170–260 by 5–6 μm
Loa loa	250–300 by 6–8.5 μm
Adult female	70 by 0.5 mm
Adult male	30–36 by 0.6 mm
Onchocerca volvulus	150–368 by 5–9 μm
Adult female	34–50 cm by 270–400 μm
Adult male	19–42 cm by 130–210 μm
Mansonella perstans	200 by 4.5 μm
Mansonella streptocerca	180–240 by 3 μm
Mansonella ozzardi	175–240 by 4.5 μm
Dracunculus medinensis	
Adult female	500–1,200 by 0.9–1.7 mm
Adult male	12–29 by 0.4 mm
Larva	500–750 by 15–25 μm
Gnathostoma spinigerum	
Adult female	25–54 mm
Adult male	11–25 mm

APPENDIX 4

Quality Control Recording Sheets

Sheets A4.1 through A4.4 are adapted from Garcia LS (section ed), Parasitology, *in* Isenberg HD, ed, *Clinical Microbiology Procedures Handbook*, 2nd ed, ASM Press, Washington, DC, 2004.

1. Reagent Name:______________________________

2. QC Requirements (frequency):________________________

3. Acceptable Criteria:____________________________

Negative Control:____________________________

Positive Control:____________________________

Date	Lot No.	Exp. Date	QC Organism	Tech	Results (A/NA)[1]	Comments Corrective Action

Comments:________________________________

____

Date	Lot No.	Exp. Date	QC Organism	Tech	Results (A/NA)	Comments Corrective Action

Comments:________________________________

____

Date	Lot No.	Exp. Date	QC Organism	Tech	Results (A/NA)	Comments Corrective Action

Comments:________________________________

____

Date	Lot No.	Exp. Date	QC Organism	Tech	Results (A/NA)	Comments Corrective Action

Comments:________________________________

____

---

[1] A = Acceptable, NA = Not Acceptable

Sheet A4.1 Diagnostic parasitology quality control (QC) (reagents).

Manufacturer/Product: ______________________________

QC Frequency: ______________________________

Acceptable (A): ______________________________

Not Acceptable (NA): ______________________________

REAGENT	LOT #	EXP. DATE

QC RESULTS	DATE	TECH	QC RESULTS	DATE	TECH

Sheet A4.2 Diagnostic parasitology quality control (QC) (reagents)—example for multiple reagents.

1. Patient's Name/Organism trying to isolate: ____________________

2. Medium Number 1: ____________________

   Medium Number 2: ____________________

3. ATCC Control Strain (Indicate Organism Name and ATCC Number): ____________________

   ____

4. Frequency: QC strain must be set up every time patient specimen is cultured (there are no exceptions to this requirement):

MEDIUM 1: ____________________

Date	Medium Lot No.	Exp. Date	Tech	A[1]	NA[2]	Date Read	Comments[3] Corrective Action

Comments: ____________________

____

____

MEDIUM 2: ____________________

Date	Medium Lot No.	Exp. Date	Tech	A[1]	NA[2]	Date Read	Comments[3] Corrective Action

Comments: ____________________

____

____

____

[1] Acceptable: QC Organism growth or motile trophozoites can be detected microscopically in sample of QC medium (inoculated with ATCC strain)

[2] Not Acceptable: QC Organism growth or motile trophozoites cannot be detected microscopically in sample of QC medium (inoculated with ATCC strain)

[3] Follow-up: In the event the QC culture is negative, the control strain should be resubbed to fresh media.

Sheet A4.3 Diagnostic parasitology quality control (QC) (culture)—example of a worksheet.

EQUIPMENT DESCRIPTION: ______________________________
(Include Inventory Number)

Serial Number: ________ **Model Number:** ________ **Purchase Date:** ________

EQUIPMENT LOCATION: ______________________________

MAINTENANCE REQUIREMENTS AND TIME FRAMES:
(List specific requirements, time frames below - use table for recording actual maintenance.)

1. ______________________________
2. ______________________________
3. ______________________________
4. ______________________________

DATE AND INITIALS	MAINTENANCE (List # of Maintenance Requirements)	COMMENTS/CORRECTIVE ACTION

REMEMBER TO ATTACH ALL RELEVANT PAPERWORK TO THIS SHEET (REPAIR INVOICES, REPLACEMENT PART INVOICES, ETC.)

Sheet A4.4 Equipment maintenance. *(continued on next page)*

DATE AND INITIALS	MAINTENANCE (List # of Maintenance Requirements)	COMMENTS/CORRECTIVE ACTION

Sheet A4.4 *(continued)*

APPENDIX 5

Commercial Supplies and Suppliers

(See Tables A5.1 to A5.7 on the following pages.)

TABLE A5.1 Sources of commercial reagents and supplies[a]

Reagent or supply	AJP Scientific	ALPHA-TEC	Avantor (J.T.Baker)	bioMérieux (PML Microbiologicals)	City Chemical Corp.	Evergreen Scientific	Fisher Scientific (Thermo Fisher Scientific)	Hardy Diagnostics	Harleco	Medical Chemical Corp.	Meridian Bioscience, Inc.	Rowley Biochem	Scientific Device Lab	Thermo Fisher Scientific (Remel)
Specimen collection kit(s)														
Formalin/PVA		X		X		X		X		X	X		X	X
PVA, modified (copper base)		X						X		X	X			X
PVA, modified (zinc base)		X		X		X		X		X	X			X
MIF	X	X						X		X	X			X
SAF		X		X		X		X		X	X		X	X
Universal Fixative[b]										X				
Pinworm paddles								X					X	
Preservatives (bulk)														
Schaudinn's fixative solution (mercury)		X+/–						X+		X+	X+	X–		X–
PVA fixative solution	X	X						X		X	X		X	X
PVA powder[c]			X				X							
MIF solution	X	X						X		X	X			
SAF solution		X		X				X						
Concentration systems													X	
Formalin-ethyl acetate		X		X		X		X		X	X			
Zinc sulfate (sp gr, 1.18/1.20)		X	X					X		X			X	

Stains													
Trichrome, solution	X(W)	X(W)		X			X(W)		X(W)	X(W)	X(W,G)	X(W)	X(W)
Trichrome, modified^{d}	X	X		X			X		X	X		X	X
Trichrome, dye powders													
Chromotrope 2R			X					X			X		
Fast green FCF			X			X		X			X		
Light green SF yellowish			X			X		X			X		
Hematoxylin, solution				X			X		X		X		
Hematoxylin, powder			X			X		X			X		
Chlorazol–black E, powder					X						X		
Giemsa, solution	X	X	X			X	X	X	X		X		X
Giemsa, powder			X			X		X			X		
Rapid blood stains									X				X
Carbol fuchsin–Kinyoun's	X	X		X		X	X	X	X		X		X
Modified acid-fast	X	X					X	X					
Modified acid-fast with DMSO		X		X									X
Calcofluor white	X					X	X						X
Auramine-rhodamine				X		X			X				X
Acridine orange				X									X
Toluidine blue O								X	X				
Miscellaneous													
Lugol's iodine, dilute 1:5	X	X		X			X	X	X	X	X		X

(continued)

TABLE A5.1 Sources of commercial reagents and supplies[a] *(continued)*

Reagent or supply	AJP Scientific	ALPHA-TEC	Avantor (J.T.Baker)	bioMérieux (PML Microbiologicals)	City Chemical Corp.	Evergreen Scientific	Fisher Scientific (Thermo Fisher Scientific)	Hardy Diagnostics	Harleco	Medical Chemical Corp.	Meridian Bioscience, Inc.	Rowley Biochem	Scientific Device Lab	Thermo Fisher Scientific (Remel)
Dobell & O'Connor's iodine		X					X	X		X			X	X
Triton X-100		X	X					X		X	X		X	X
Eosin/saline solution (1%)							X			X				
Mayer's albumin		X						X						X
Control slides or suspensions		X		X				X			X		X	X
Alcohols, xylene														
Methanol (thin blood film)	X			X			X	X		X		X		X
Ethanol 70%	X	X					X	X		X		X		X
Ethanol 90%		X					X							X
Ethanol 95%	X	X					X	X				X		
Ethanol absolute							X (200 proof)	X		X				
Xylene	X						X	X		X		X		
Carbol-xylene	X						X	X						
Xylene substitute		X					X	X		X				X

[a] NOTE: Since products can frequently vary, please consult the vendor websites and representatives for current product availability and pricing.

Abbreviations: DMSO, dimethyl sulfoxide; G, Gomori; W, Wheatley; +, with acetic acid; –, without acetic acid.

[b] Contains no mercury, formalin, or polyvinyl alcohol (PVA) (TOTAL-FIX).

[c] Use grade with high hydrolysls and low viscosity for parasite studies.

[d] Used for the identification of microsporidia spores in stool or other specimens.

TABLE A5.2 Addresses of suppliers listed in Table A5.1

AJP Scientific, Inc. (Eng Scientific)
P.O. Box 1589
Clifton, NJ 07015
(800) 922-0223
(973) 472-7200
E-mail: info@engscientific.com
Products also available through various distributors

ALPHA-TEC Systems, Inc.
P.O. Box 5435
Vancouver, WA 98668
(800) 221-6058
(360) 260-2779
Fax: (360) 260-3277
Website: www.alphatecsystems.com
Products also available through various distributors

Avantor Performance Materials
(formerly J.T. Baker/Mallinckrodt Baker)
3477 Corporate Parkway
Center Valley, PA 18034
(610) 573-2600
Fax: (610) 57302610

bioMérieux, Inc.
(PML Microbiologicals)
100 Rodolphe Street
Durham, NC 27712
(800) 682-2666

City Chemical Corp.
139 Allings Crossing Road
West Haven, CT 06516
(800) 248–2436
(203) 932–2489
E-mail: sales@citychemical.com
Chlorazol black E stain

Evergreen Scientific
2300 East 49th Street
P.O. Box 58248
Los Angeles, CA 90058-0248
(800) 421-6261
(800) 372-7300 in California
(213) 583-1331
Fax: (213) 581-2503
Website: www.evergreensci.com
Products also available through various distributors

Fisher Scientific (Thermo Fisher Scientific)
2761 Walnut Avenue
Tustin, CA 92681
(800) 766-7000 (national no.)
Fax: (800) 926-1166 (national no.)
Website: www.fishersci.com
Products available through regional sales offices

Hardy Diagnostics
1430 West McCoy Lane
Santa Maria, CA 93455
(800) 266-2222
Fax: (805) 346-2760
Website: www.hardydiagnostics.com
E-mail: sales@hardydiagnostics.com
Products also available through various distributors

Harleco
Voigt Global Distribution LLC
P.O. Box 412762
Kansas City, MO 64141–2762
(877) 484–3552
Website: www.vgdusa.com
Products available through American Scientific Products; product numbers remain unchanged

Medical Chemical Corp.
19430 Van Ness Avenue
Torrance, CA 90501
(800) 424-9394
Fax: (310) 787-4464
Website: www.med-chem.com
Products also available through various distributors

Meridian Bioscience, Inc.
3741 River Hills Drive
Cincinnati, OH 45244
(800) 543-1980
(513) 271-3700
Fax: (513) 271-0124
Website: www.meridianbioscience.com
Parasite Panels (teaching specimens)

Rowley Biochemical, Inc.
10 Electronics Avenue
Danvers, MA 01923
(978) 739–4883
Fax: (978) 739-5640
Website: www.rowleybio.com
Products available only through company

Scientific Device Laboratory, Inc.
411 East Jarvis Avenue
Des Plaines, IL 60018
(847) 803-9495
Fax: (847) 803-8251
Website: www.scientificdevice.com
Products also available through various distributors
Cyclospora cayetanensis teaching slides available

Thermo Fisher Scientific (Remel)
12076 Santa Fe Drive
Lenexa, KS 66215
(800) 255-6730
Fax: (800) 477-5781
Website: www.remelinc.com
Products available through other distributors

TABLE A5.3 Sources of available reagents for immunodetection of parasitic organisms or antigens[a]

Company	*Cryptosporidium* EIA or IFA	*Cryptosporidium-Giardia* combination (various methods)	*Entamoeba histolytica* EIA	*E. histolytica/ E. dispar* group EIA	*Giardia* EIA or IFA	*Crypto-Giardia* cartridge and/ or separate cartridges	*Crypto-Giardia-E. histolytica/ E. dispar* group cartridge	*Trichomonas vaginalis* (various methods)	Other parasites (methodology varies, often EIA)	Water-testing reagents
Alere (Wampole, Inverness Medical)									Binax NOW Malaria, Clearview Malaria Combo, Rapid Toxoplasma Ab	
Becton-Dickinson								X (DNA Probe) (Affirm VP III) (*Trichomonas*, *Gardnerella*, *Candida*)		
Alere (Biosite Diagnostics, Inc.)							X (Triage Parasite Panel)			
BioFire		FilmArray Gastrointestinal panel (also includes *Cyclospora*)	FilmArray Gastrointestinal panel							
Cellabs	X (IFA)	X (DFA)			X (IFA, EIA)				Filariasis Ab *Leishmania* Ag, Ab *Plasmodium* Ag, Ab *T. cruzi* Ab *Toxocara* Ab	

Hologic GenProbe							Aptima *Trichomonas* amplification assay	
Luminex, Inc.		xTAG[b]						
Medical Chemical Corp.	X	X (DFA)			X			
Meridian Diagnostics, Inc.	X	X (DFA)			X	X		
Remel (Thermo Fisher Scientific)	X	X (EIA)		X	X	X		
Sekisui Diagnostics (Genzyme Diagnostics)							OSOM Trichomonas rapid test	
TechLab, Inc./Alere	X	X (DFA, EIA, Rapid)	X		X			
Waterborne, Inc.								X

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[a] Tests for the *Entamoeba histolytica*/*E. dispar* group or *E. histolytica* require the use of fresh or fresh, frozen stools or those submitted in Cary-Blair. All other tests (with the exception of DFA for *Cryptosporidium*/*Giardia* combination) can be used with fresh or preserved stools (formalinized base, NOT PVA; some single-vial systems may be acceptable [Universal Fixative]; consult manufacturer). Since the combination *Cryptosporidium-Giardia* tests (DFA) are based on visual recognition of the fluorescent oocysts and/or cysts, the specimens must not be frozen if fresh stools are used for testing; it is also recommended that these FA tests be performed on centrifuged stool sediment to enhance sensitivity.

[b] xTAG, multiplexed nucleic acid test that simultaneously detects 11 common viral, bacterial, and parasitic causes of infectious gastroenteritis from a single patient sample.

TABLE A5.4 Addresses of suppliers listed in Table A5.3

Alere, Inc.
51 Sawyer Road, Suite 200
Waltham, MA 02453-3448
Client Service: (877) 441-7440 (option 1)
Technical Service: (877) 441-7440 (option 2)

MicroProbe Corp. (Becton Dickinson Affirm VPIII)
1725 220th St. NE
Bothell, WA 98021
(*Trichomonas*, *Gardnerella*, *Candida*)
(201) 847-6800
Website: www.bd.com

Alere (Biosite Diagnostics, Inc.)
9975 Summers Ridge Road
San Diego, CA 92121
(858) 455-4808
(888) BIOSITE
Fax: (858) 455-4815
Website: www.biosite.com

BioFire Diagnostics, Inc.
390 Wakara Way
Salt Lake City, UT 84108
(801) 736-6354
www.BioFireDX.com

Cellabs
P. O. Box 421, Brookvale, NSW
2100 Australia
enquiries@cellabs.com.au

Hologic Gen-Probe, Inc.
10210 Genetic Center Drive
San Diego, CA 92121
Customer Service: (800) 523-5001 (press 1)
Technical Support: (888) 484-4747
webmaster@gen-probe.com

Luminex, Austin
12212 Technology Blvd.
Austin, TX 78727
(888) 219-8020
orders@luminexcorp.com

Medical Chemical Corp.
19430 Van Ness Avenue
Torrance, CA 90501
(800) 424-9394
Fax: (310) 787-4464
Website: www.med-chem.com

Meridian Bioscience, Inc.
3471 River Hills Dr.
Cincinnati, OH 45244
(513) 271-3700
Fax: (513) 271-0124
Website: www.meridianbioscience.com

Remel (Thermo Fisher Scientific)
12076 Santa Fe Drive
Lenexa, KS 66215
(800) 255-6730
Fax: (800) 477-5781
Website: www.remelinc.com

American Diagnostica Inc. (Sekiui Medical Co., Ltd.)
500 West Avenue
Stamford, CT 06902
(203) 602-7777
Fax: (203) 602-2221
http://www.americandiagnostica.com

TechLab, Inc. (Alere)
2001 Kraft Dr.
Blacksburg, VA 24060-6358
(800) 832-4522
(540) 953-1664
Fax: (540) 953-1665
Website: www.techlab.com

Waterborne, Inc.
6945 Hurst Street.
New Orleans, LA 70115
(866) 895-3338
contact@waterborneinc.com

TABLE A5.5 Commercial suppliers of diagnostic parasitology products[a]

Abbott Laboratories
100 Abbott Park Rd.
Abbott Park, IL 60064-3500
(847) 937-6100
Website: www.abbott.com
Toxoplasma (EIA for IgG, IgM) (Architect)

ALPHA-TEC Systems, Inc.
P.O. Box 5435
Vancouver, WA 98668
(800) 221-6058
(360) 260-2779
Fax: (360) 260-3277
Website: www.alphatecsystems.com
Customer Service: CustSvc@AlphaTecSystems.com
Technical Support: Technical@AlphaTecSystems.com
Control slides, reagents, stains (including polychrome IV), collection systems, concentration systems, QC suspensions

Bayer Diagnostics
511 Benedict Ave.
Tarrytown, NY 10591
(800) 242-2787
Website: www.bayer.com
Toxoplasma (EIA for IgG and IgM)

Beckman Coulter
4300 N. Harbor Blvd.
Fullerton, CA 92834
(800) 233-4685
Fax: (800) 643-4366
Website: www.beckman.com
Toxoplasma (EIA for IgG and IgM)

Becton Dickinson Advanced Diagnostics
Becton Dickinson Europe
2350 Qume Dr.
San Jose, CA 95131-1087
(877) 232-8995, Operator 5
Fax: (800) 954-2347
Website: www.bdfacs.com
Malaria P.f. ParaSight F dipstick
Genzyme ColorPAC *Giardia/Cryptosporidium* rapid assay

BioFire Diagnostics, Inc.
390 Wakara Way
Salt Lake City, UT 84108
(801) 736-6354
www.BioFireDX.com
FilmArray gastrointestinal panel (*Giardia/Cryptosporidium/Cyclospora/Entamoeba histolytica*)

Biokit USA, Inc.
113 Hartwell Ave.
Lexington, MA 02173
(800) 926-2253
Fax: (617) 861-4065
Website: www.biokitusa.com
Toxoplasma (LA for IgG)

BIOMED Diagnostics
PO Box 2366
White City, OR 97503
(800) 964-6466
Fax: (541) 830-3001
Website: www.biomeddiagnostics.com
InPouch TV (visual identification and culture system for *Trichomonas vaginalis*)

Biosite Diagnostics, Inc. (Alere)
9975 Summers Ridge Road
San Diego, CA 92121
(858) 455-4808
Fax: (858) 455-4815
Website: www.biosite.com
Triage Parasite Panel-Rapid (*Cryptosporidium*, *Giardia*, *Entamoeba histolytica/E. dispar*)

BORDIER Affinity Products SA
Chatanerie 2
CH-1023 Crissier, Switzerland
+41 21 633 31 67
Fax: +41 21 633 31 78
cb@bordier.ch
Antigen detection (*Enterocytozoon bieneusi/Encephalitozoon intestinalis*)
Antibody detection (toxocariasis, hydatid disease, filariasis, strongyloidiasis, schistosomiasis, visceral leishmaniasis, amebiasis)

Core Diagnostics
Aspect Court, 4 Temple Road
Birmingham, B2 5HG UK
44-121-222-2523
sales@corediag.com
Infectious disease rapid screening

Siemens Medical Solutions Diagnostics (formerly Diagnostic Products Corp.)
5700 W. 96th St.
Los Angeles, CA 90045-5597
(310) 645-8200
Fax: (310) 645-9999
Website: www.biospace.com
www.healthcare.siemens.com
Toxoplasma (EIA, Immulite, automated)

DiaSorin
1990 Industrial Blvd.
P.O. Box 285
Stillwater, MN 55082
(800) 328-1482
Fax: (612) 779-7847
Website: www.diasorin.com
Toxoplasma (EIA for IgG and IgM)

(continued)

TABLE A5.5 Commercial suppliers of diagnostic parasitology products[a] *(continued)*

DIASYS Ltd.
Unit 5 Sapphire Centre
Fishponds Road
Wokingham
Berkshire RG41 2QL UK
Website: www.diasyscorp.com
Email: sales@diasys.com
Semiautomated stool concentrate examination system; antigen detection (malaria); antibody detection (*Leishmania*)

Eastman Kodak Co.
Rochester, NY 14650
(800) 225-5352
(716) 458-4014
Products available only through American Scientific Products, Fisher Scientific, VWR, and company PVA powder, Giemsa powder

Evergreen Scientific
2300 East 49th St.
Los Angeles, CA 90058
(800) 421-6261
(213) 583-1331
Fax: (213) 581-2503
Website: www.evergreensci.com
Stool collection system, concentration system, pinworm collection

Flow Inc.
6127 SW Corbett
Portland, OR 97201
(503) 246-2710
Fax: (503) 245-7666
Website: www.malariatest.com
E-mail: MIKEatFLOW@aol.com
OptiMAL

GenBio
15222-A Avenue of Science, Suite A
San Diego, CA 92128
(800) 288-4368
Fax: (858) 592-9400
Website: www.genbio.com
Toxoplasma (EIA and IFA for IgG and IgM)
TORCH PANEL

Hardy Diagnostics
1430 West McCoy Ln.
Santa Maria, CA 93455
(800) 266-2222
Fax: (805) 346-2760
Website: http://hardydiagnostics.com
Stains, reagents, collection system, concentration systems, control slides

HDC Corp.
2109 O'Toole Ave., Suite M
San Jose, CA 95131
(408) 954-1909
Fax: (408) 954-0340
Website: www.hdccorp.com
Entero-Test capsules (adult and pediatric) (method of sampling upper gastrointestinal tract)

Hologic Gen-Probe, Inc.
10210 Genetic Center Drive
San Diego, CA 92121
Customer Service: (800) 523-5001 (press 1)
Technical Support: (888) 484-4747
Website: www.hologic.com/
Aptima *Trichomonas vaginalis*

Hemagen Diagnostics
34–40 Bear Hill Rd.
Waltham, MA 02154
(800) 436-2436
Fax: (781) 890-3748
Website: www.hemagen.com
Chagas' disease (EIA), *Toxoplasma* (EIA, IFA)

Immunetics
63 Rogers St.
Cambridge, MA 02142
(617) 492-5416
Fax: (617) 868-7879
Website: www.immunetics.com
Babesia (IB),[b] Chagas' disease (IB),[b] cysticercosis (IB),[b] echinococcosis (IB),[b] *Leishmania* (IB)[b]

Interfacial Dynamics Corp.
17300 SW Upper Boones Ferry Rd., Suite 120
Portland, OR 97224
(503) 256-0076
(800) 323-4810
Fax: (503) 255-0989
E-mail: idclatex@teleport.com
Uniform-sized polystyrene microspheres (can be used to check microscope calibrations)

IVD Research Inc.
5909 Sea Lion Place, Suite D
Carlsbad, CA 92008
(760) 929-7744
Fax: (760) 431-7759
Website: www.ivdresearch.com, www.safepath.com
SafePath offers numerous assays for veterinary, environmental, and food safety applications
Amebiasis (EIA), cysticercosis (EIA), echinococcosis (EIA), *Toxocara* (EIA), trichinosis (EIA), *Toxoplasma* (EIA); check for FDA approval

Luminex, Austin
12212 Technology Blvd.
Austin, TX 78727
(888) 219-8020
orders@luminexcorp.com
Molecular, automated ID panel includes *Giardia/Cryptosporidium*

(continued)

TABLE A5.5 *(continued)*

Medical Chemical Corp.
19430 Van Ness Avenue
Torrance, CA 90501
(800) 424-9394
Fax: (310) 787-4464
Website: www.med-chem.com
Reagents, modified trichrome stain for microsporidia, other stains, collection system (including new TOTAL-FIX fecal preservative [no mercury, no formalin, no PVA]), concentration system, parasitology website (current information, photographs, commonly asked questions, case histories)

Launch Diagnostics Limited
Ash House, Ash Road
New Ash Green, Longfield
Kent, DA3 8JD UK
enquiries@launchdiagnostics.com
Antibody detection (hydatid disease, toxoplasmosis, other related reagents)

Meridian Bioscience, Inc.
3741 River Hills Dr.
Cincinnati, OH 45244
(800) 543-1980
(513) 271-3700
Fax: (513) 271-0124
Stains, reagents, collection system, concentration system, *Cryptosporidium* spp. (EIA, FA), *Giardia* spp. (EIA, FA)

Oxoid Limited (Thermo Fisher)
Wade Road
Basingstoke, Hampshire
RH24 8PW UK
oxoid.info@thermofisher.com
Antigen detection (*Cryptosporidium*, *Entamoeba histolytica*, *Giardia*); stain controls (*Cryptosporidium*, microsporidia, protozoa)

PML Microbiologicals
27120 SW 95th Ave.
Wilsonville, OR 97070
(800) 547-0659
(503) 639-1500 in Oregon
Fax: (800) 765-4415
Website: www.pmlmicro.com
Stains, reagents, collection system, concentration system, control slides

QBC Diagnostics (The Drucker Company)
168 Bradford Drive
Port Matilda, PA 16870
814-692-7661
Fax: (814) 692-7662
Website: www.qbcdiagnostics.com
QBC Malaria test

Scientific Device Laboratory, Inc.
411 E. Jarvis Ave.
Des Plaines, IL 60018
(847) 803-9495
Fax: (847) 803-8251
Website: www.scientificdevice.com
Stains, stain kit, modified trichrome stain for microsporidia, fixative (formalin free), formalin-fixed protozoa and helminth eggs and larvae, control slides (*Cryptosporidium*, *Cytoisospora*, microsporidia, and *Pneumocystis*), stained slides

Sekisui Diagnostics
3463 Magic Dr., Suite 360
San Antonio, TX 78229
(210) 582-5838
Fax: (210) 582-5839
www.genzymediagnostics.com
OSOM *Trichomonas* rapid test for antigen

TechLab
2001 Kraft Drive
Blacksburg, VA 24060-6358
(540) 953-1664
Fax: (540) 953-1665
Website: www.techlab.com
E-mail: techlab@techlab.com
EIA antigen detection (*Giardia*, *Cryptosporidium*, *Entamoeba histolytica*, *Giardia-Cryptosporidium*)

Thermo Scientific (Remel)
12076 Santa Fe Dr.
Lenexa, KS 66215
(800) 255-6730
Fax: (800) 477-5781
Website: www.remelinc.com
Stains, reagents, collection system, concentration system, control slides

Volu-Sol, Inc. (a division of Biomune, Inc.)
5095 West 2100 South
Salt Lake City, UT 84120
(800) 821-2495
(801) 974-9474
Fax: (800) 860-4317
Website: www.volusol.com
Parasitology starter kit, stains, reagents, control slides

VWR Scientific, Inc.
P.O. Box 7900
San Francisco, CA 94120
(415) 468-7150 in Northern California
(213) 921-0821 in Southern California
(rest of United States, use either number)
Stains, reagents, collection system

(continued)

TABLE A5.5 Commercial suppliers of diagnostic parasitology products[a] *(continued)*

Wampole Lab (Inverness Medical) P.O. Box 1001 Cranbury, NJ 08512 (800) 257-9525 Fax: (800) 532-0295 Website: www.invernessmedicalpd.com EIA antigen detection (*Entamoeba histolytica*, *Giardia*, *Cryptosporidium*)	**Zenyth Therapeutics Limited** 576 Swan Street Richmond, Victoria 3121 Australia 61 3 9208 4000 Fax: 61 3 9208 4356 Website: www.zenyth.com.au Antigen detection (rapid or dipstick) for filariasis, malaria, and *P. falciparum* malaria

[a] Modified from Leber A (ed), *Clinical Microbiology Procedures Handbook*, 4th ed, ASM Press, Washington, DC, in press. Much of the updated immunology testing information was provided by Marianna Wilson (CDC). Abbreviations: DFA, direct fluorescent-antibody assay; EIA, enzyme immunoassay; IB, immunoblot; IFA, indirect fluorescent-antibody assay; IgG, immunoglobulin G; LA, latex agglutination; PVA, polyvinyl alcohol; QC, quality control; Rapid, rapid immunochromatographic.

[b] Not cleared by the Food and Drug Administration for *in vitro* diagnostic use.

TABLE A5.6 Sources of additional teaching materials, including case histories (see also chapter 12)

Centers for Disease Control and Prevention MS-F36 4770 Buford Highway NE Atlanta, GA 30341-3724 (404) 639-3331 (800) 311-3435 Website: www.dpd.cdc.gov/DPDx/HTML/Contactus.htm Extensive training materials, including the DPDx CD-ROM disk; CDC Public Health Image Library Bench Aids for teaching	Medical Chemical Corp. 19430 Van Ness Avenue Torrance, CA 90501 (800) 424-9394 Fax: (310) 787-4464 Website: www.med-chem.com Parasitology website (current information, photographs, commonly asked questions, case histories) (developed by Lynne Garcia)

TABLE A5.7 Sources of parasitologic specimens (catalogs of available materials and price lists available from the companies listed)[a]

ALPHA-TEC Systems, Inc.
P.O. Box 5435
Vancouver, WA 98668
(800) 221-6058
(360) 260-2779
Fax: (360) 260-3277
Website: www.alphatecsystems.com
Control slides and suspensions

Carolina Biological Supply Co.
2700 York Road
Burlington, NC 27215
(800) 334-5551
(919) 584-0381
Fax: (800) 222-7112
Website: www.carolina.com
Individual slides, slide sets, CDM disk and slides

McGill University Centre for Tropical Diseases
National Reference Centre for Parasitology
Montreal General Hospital
Research Institute room R3-137
1650 Cedar Avenue
Montreal, Quebec H3G 1A4
Canada
(514) 934-8049
Fax: (514) 934-8347
Website: www.medicine.mcgill.ca/tropmed/txt/sales.htm
Contact: evelyne.kokoskin@mcgill.ca
Teaching specimens: formalin, blood films, teaching sets

Medical & Science Media
P.O. Box 3224
Mt. Druitt Village, NSW
Australia
61 2 9675 7750
Fax: 61 2 9675 7702
Website: www.msmedia.com.au
Extensive lists of slides, photomicrographs, CD-ROM disks

Meridian Bioscience, Inc.
Proficiency & Controls Department
3741 River Hills Drive
Cincinnati, OH 45244
(800) 543-1980, ext. 335
(513) 271-3700, ext. 335
Fax: (513) 271-0124
Website: www.meridianbioscience.com
E-mail: jross@meridianbioscience.com
Parasite Panels (teaching specimens) formalin, slides (protozoa, helminths, blood parasites)

Scientific Device Laboratory, Inc.
411 E. Jarvis Avenue
Des Plaines, IL 60018
(800) 448-4855
(708) 803-9495
Fax: (708) 803-8251
Website: www.scientificdevice.com
Control slides and/or suspensions, *Cyclospora cayetanensis* teaching slides

Ward's Natural Science Establishment, Inc.
P.O. Box 92912
Rochester, NY 14692
(800) 962-2660
Website: www.wardsci.com
Slide sets, but availability may be a problem

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[a] Always ask to see a sample slide or specimen prior to ordering; quality varies tremendously.

APPENDIX 6

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WEBSITES

Parasite Images

Do a web search under general parasite categories and/or specific genus/species of parasites, then select "images"; many images will appear. However, you may see the same image under different sites/links; therefore, it is difficult to determine which site is the original. This impacts your ability to use the images due to possible copyright issues.

Parasitology Information

Centers for Disease Control and Prevention:
www.dpd.cdc.gov/dpx/
Medical Chemical Corporation:
www.med-chem.com

Literature

NCBI National Library of Medicine (PubMed):
http://www.ncbi.nlm.nih.gov/pubmed

Government Websites Related to Health Care Regulations

Centers for Medicare and Medicaid Services (CMS):
http://cms.hhs.gov/
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Compliance Program, Fraud Alerts, Advisory Opinions, Red Book, Work Plan:
http://oig.hhs.gov/
Medicare Learning Network:
http://www.cms.gov/Outreach-and-Education/Medicare-Learning-Network-MLN/MLNGenInfo/
National Technical Information Service:
www.ntis.gov
National Center for Health Statistics:
http://www.cdc.gov/nchs/about.htm
Office of the Inspector General (OIG) Compliance
Documents for Clinical Laboratories, Hospitals, and Third Party Billing:
http://www.gpo.gov/fdsys/search/search.action?st=Compliance+document+for+clinical+laboratories%2C+hospitals+and+%22third+party%22+billing

APPENDIX 7

“Late-Breaking” Published Information

The following information was published in the last couple of months of 2014 and the first few months of 2015 and contains relevant information for the field of diagnostic medical parasitology. This is “late-breaking” published information that may be helpful for the reader.

Intestinal Protozoa (Amebae)

Entamoeba spp.

Petropolis DB, Faust DM, Deep Jhingan G, Guillen N. 2014. A new human 3D-liver model unravels the role of galectins in liver infection by the parasite *Entamoeba histolytica*. *PLoS Pathog* **10:**e1004381. doi:10.1371/journal.ppat.1004381.

The authors developed a human three-dimensional (3D) liver *in vitro* model using cultured liver sinusoidal endothelial cells and hepatocytes in a collagen-I matrix. They found important hepatic markers and showed that the cell layers act as a biological barrier. Invasion of *Entamoeba histolytica* was investigated using wild-type strains and amebae with different virulence or adhesive properties. They demonstrated that amebic Gal/GalNAc lectin influenced the dependence of the endothelium crossing. This 3D liver model supported the molecular analysis of human cellular responses and showed for the first time the importance of the role of human galectins in the adhesion of parasites to the endothelial cells. **The presence of galectin-1 and -3 in the extracellular medium led to proinflammatory cytokine release, thus supporting the role for human galectins’ hepatic inflammatory response.** The approach in this study using a 3D liver model may be relevant for other parasitic or viral hepatic infections.

Blastocystis spp.

Bálint A, Dóczi I, Bereczki L, Gyulai R, Szücs M, Farkas K, Urbán E, Nagy F, Szepes Z, Wittmann T, Molnár T. 2014. Do not forget the stool examination!: cutaneous and gastrointestinal manifestations of *Blastocystis* sp. infection. *Parasitol Res* **113:**1585–1590.

Blastocystis spp. are the most common parasite in the human intestinal tract worldwide. The infection is characterized by diarrhea and abdominal pain; however, extraintestinal findings such as skin lesions may also be present. This study was designed to assess the frequency, clinical symptoms, and skin manifestations of confirmed positive *Blastocystis* spp. infections. Eighty patients were assessed retrospectively. The average age of the patients was 46.3 years. The number of female patients was higher than the number of males (48 versus 32; 60 versus 40%). Gastrointestinal and dermatological symptoms, biochemical tests, and hematological tests were included in the study. The skin manifestations were analyzed using descriptions, photos, and biopsies. Patients (11.25%) exhibited skin manifestations associated with *Blastocystis* spp., mainly seen in females. *Blastocystis* spp. were found in 6% of symptomatic patients between 2005 and 2013. Of the 80 patients with confirmed infection, 73.75% had gastrointestinal symptoms: 40 patients had abdominal pain and 17 had blood in their stool; other symptoms, such as excess gas (15), weight loss (8), perianal pain or itching (6), passing stool with mucus (5), vomiting (2), and fever (2), were less common. Abdominal pain in the group without skin lesions was higher than in patients with skin problems ($P = 0.007$). The mean value of C-reactive protein demonstrated elevated levels, but eosinophils were normal. Also, there were no significant differences in eosinophilia between patients with versus without skin manifestations. **Based on these results, the authors suggest a stool parasite examination for patients with skin lesions of unknown origin.**

Ruaux CG, Stang BV. 2014. Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. *PLoS One* **9:**e107496. doi:10.1371/journal.pone.0107496.

It has been demonstrated that domestic dogs and cats can be infected with a number of protozoan enteric parasites, including

Blastocystis spp. For this reason, there is increased interest in *Blastocystis* as a potential enteric pathogen and in any possible role dogs and cats may play in human infections. Fecal specimens were collected from 103 shelter-resident dogs, 105 shelter-resident cats, 51 client-owned dogs, and 52 client-owned cats. *Blastocystis* was detected and subtypes identified using a nested PCR. Shelter animals were much more likely to test positive for *Blastocystis* ($P < 0.05$ for dogs, $P = 0.009$ for cats). Sequence analysis of these animals confirmed that they were carrying a number of *Blastocystis* subtypes. There was no relationship between *Blastocystis* infection and gastrointestinal symptoms in either dogs or cats. **These data suggest that shelter-companion dogs and cats are more likely to be positive for *Blastocystis* spp.** However, the data also suggest that *Blastocystis* spp. are unlikely to be major enteric pathogens in these species.

Fréalle E, El Safadi D, Cian A, Aubry E, Certad G, Osman M, Wacrenier A, Dutoit E, Creusy C, Dubos F, Viscogliosi E. 2015. Acute blastocystis-associated appendicular peritonitis in a child, Casablanca, Morocco. *Emerg Inf Dis* **21:**91–94.

This represents another case in which a *Blastocystis* sp. has been implicated as a disseminated infection (lumen, mucosa) associated with acute inflammation of the appendix. Although 26 other family members reported cases of acute to moderate gastroenteritis, diagnostic parasitology testing was not performed; thus, the definitive diagnosis in these individuals was not confirmed.

Intestinal Protozoa (Flagellates and Ciliates)

Dientamoeba fragilis

Stark D, Garcia LS, Barratt JL, Phillips O, Roberts T, Marriott D, Harkness J, Ellis JT. 2014. Description of *Dientamoeba fragilis* cyst and precystic forms from human samples. *J Clin Microbiol* **52:**2680–2683.

Dientamoeba fragilis is a common pathogenic protozoan of humans and has been reported worldwide. Although the cyst stage of the parasite has been described in an animal model, no cyst stage in humans had been described in detail from clinical samples. **In this study, the authors describe the precyst and cyst forms from human clinical samples. In light of the fact that *D. fragilis* infection can cause chronic long-term infections, these cyst forms may be involved in autoinfection.** The nuclear structure is identical to that found in *D. fragilis* trophozoites. All cysts identified were binucleate, with each nucleus containing the large central karyosome and a delicate nuclear membrane. The nucleus is often fragmented into separate chromatin granules, and chromatin is usually not seen on the nuclear membrane. However, these "true" cysts are rarely seen and identified in clinical samples. The precystic forms were more commonly seen, with a prevalence of up to 5% in human clinical specimens. This precystic form has a compact spherical shape and is up to 50% smaller than "normal" trophozoites. The precysts range in size from 4 to 5 µm, and the cytoplasm is denser and more darkly staining than is seen in normal trophozoites. Also, the cytoplasm rarely contains any inclusions.

Giardia lamblia

Liao JY, Guo YH, Zheng LL, Li Y, Xu WL, Zhang YC, Zhou H, Lun ZR, Ayala FJ, Qu LH. 2014. Both endo-siRNAs and tRNA-derived small RNAs are involved in the differentiation of primitive eukaryote *Giardia lamblia*. *Proc Natl Acad Sci USA* **111:**14159–14164.

In eukaryotes, small RNAs (sRNAs) including microRNAs and endogenous siRNAs (endo-siRNAs) regulate most biologic processes such as cell division and differentiation. The authors deep-sequenced the sRNA transcriptome of four different stages in the differentiation of *Giardia lamblia*, a very primitive eukaryote. They identified a large number of endo-siRNAs and found that they were produced from live telomeric retrotransposons and three genomic regions (i.e., endo-siRNA-generating regions [eSGRs]). Gradual upregulation of endo-siRNAs in the differentiation of *Giardia* suggests involvement in this process regulation. The authors identified five new kinds of tRNA-derived sRNAs. They also found that the biogenesis in four might correlate with previously discovered stress-induced tRNA-derived RNA (sitRNA). **These studies demonstrate a complex group of sRNAs in *G. lamblia* and further clarify the origin and evolution of eukaryotic sRNAs.**

Intestinal Protozoa (Coccidia and Microsporidia)

Cryptosporidium spp.

Yu Y, Zhang H, Guo F, Sun M, Zhu G. 2014. A unique hexokinase in *Cryptosporidium parvum*, an apicomplexan pathogen lacking the Krebs cycle and oxidative phosphorylation. *Protist* **165:**701–714.

It is well known that *Cryptosporidium parvum* may cause untreatable infections in patients with AIDS. This parasite has also been identified as one of the top four diarrheal pediatric pathogens in developing countries. *Cryptosporidium* is different from other apicomplexans such as *Plasmodium* and *Toxoplasma*, since it lacks many metabolic pathways including the Krebs cycle and cytochrome-based respiratory chain and depends on glycolysis for the production of ATP. This study reports on molecular and biochemical characteristics of a hexokinase in *C. parvum* (CpHK). Phylogeny suggests that apicomplexan hexokinases are highly divergent from those of humans and animals. CpHK differs from those in mammals and *Toxoplasma gondii* (TgHK) by preferring various hexoses and the capacity to use ATP and other NTPs. **While the exact action of 2-deoxy-D-glucose on *Cryptosporidium* requires confirmation, these data suggest that CpHK and the glycolytic pathway provide potential options for the development of anticryptosporidial therapeutics.**

Chandra V, Torres M, Ortega YR. 2014. Efficacy of wash solutions in recovering *Cyclospora cayetanensis*, *Cryptosporidium parvum*, and *Toxoplasma gondii* from basil. *J Food Prot* **77:**1348–1354.

It has been well documented that parasitic infections can originate from the ingestion of contaminated raw or processed fresh produce (herbs and fruits). Method sensitivity for organism detection on fresh produce is partially dependent on the effectiveness of the wash solutions used. In this study, six wash solutions (sterile E-Pure water, 3% levulinic acid–3% sodium dodecyl sulfate, 1 M glycine, 0.1 M phosphate-buffered saline, 0.1% Alconox, and 1% HCl-pepsin) were evaluated for their ability to remove *Cyclospora cayetanensis*, *Cryptosporidium parvum*, and *Toxoplasma gondii* from basil. Oocysts (100 or 1,000) were inoculated onto the upper surfaces of 25 g of basil leaves, placed in stomacher bags, and stored for 1 h at 21°C or 24 h at 4°C. Leaves were then hand washed in each solution for 1 min. DNA from the various wash solutions was extracted and amplified using PCR for parasite detection. Oocysts (1,000 oocysts per 25 g of basil) were detected in all wash solutions. At a concentration of 100 oocysts per 25 g, oocysts were found in 18.5 to 92.6% of the solutions. The lowest variability in oocyst recovery inoculated with 100 oocysts was seen in the 1% HCl-pepsin wash solution. Oocyst recovery was greater at 1 h than at 24 h postinoculation. **This study provides guidance for the use of wash solutions for produce, providing the most efficient retrieval of parasite oocysts.**

Ryan U, Fayer R, Xiao L. 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology* **141:**1667–1685.

Cryptosporidium is widely recognized as a major cause of diarrhea in developing countries. Since treatment options are very limited, control depends on an awareness of the organism biology and transmission. **Currently, 26 species are recognized on the basis of morphological, biological, and molecular studies. Of those reported in humans, *Cryptosporidium hominis* and *Cryptosporidium parvum* are responsible for the most infections. Livestock, particularly cattle, serve as an important reservoir of zoonotic infections.** However, research limitations include the lack of subtyping information for many veterinary *Cryptosporidium* species. There is limited understanding regarding host specificity of *Cryptosporidium*. The impact of climate change is also not well defined regarding organism transmission.

Sponseller JK, Griffiths JK, Tzipori S. 2014. The evolution of respiratory cryptosporidiosis: evidence for transmission by inhalation. *Clin Microbiol Rev* **27:**575–586.

Cryptosporidium infects all major vertebrates and causes significant diarrhea in humans. Children and immunodeficient individuals are more at risk for cryptosporidiosis, especially in developing countries, where high morbidity and mortality figures are seen in preschool-age children. Unfortunately, no antiprotozoal agent or vaccine exists for treatment or prevention. Respiratory cryptosporidiosis has been described in birds and mammals, including immunocompromised humans. **Respiratory cryptosporidiosis may also be seen in immunocompetent children with cryptosporidial diarrhea and unexplained cough. In addition to the traditional fecal-oral route of infection, data from animal studies, human clinical case reports, and epidemiological studies suggest respiratory transmission via secretions.** Transmission of *Cryptosporidium* oocysts may occur through inhalation or by contact with fomites contaminated by coughing, particularly since the oocysts have been found to survive for some time on fomites. Clarifying the potential role of the respiratory tract in the transmission of *Cryptosporidium* may help expand the current guidelines for disease prevention.

Chappell CL, Okhuysen PC, Langer-Curry RC, Lupo PJ, Widmer G, Tzipori S. 2015. *Cryptosporidium muris*: infectivity and illness in healthy adult volunteers. *Am J Trop Med Hyg* **92:**50–55.

Although human infections with *Cryptosporidium muris* have been reported in the literature, no definitive information has been available on clinical outcomes in healthy individuals. In this study, six healthy adult volunteers were challenged with 10^5 viable oocysts and monitored for clinical symptoms. All volunteers became infected, two suffered diarrhea, and three shed oocysts for 7 months (a longer time frame than reported with other species and volunteer infections). **Thus, it is clear that healthy individuals are susceptible to infection with *C. muris*, which causes symptoms and can become a chronic infection for many months.**

Microsporidia

Santiana M, Pau C, Takvorian PM, Cali A. 2014. Analysis of the beta-tubulin gene and morphological changes of the microsporidium *Anncaliia algerae* both suggest albendazole sensitivity. *J Eukaryot Microbiol* **62:**60–68.

***Anncaliia algerae*, an obligate intracellular microsporidian parasite, is an opportunistic human pathogen;** however, therapy has not been assessed. Albendazole is an antitubulin polymerization drug used for helminth infections and is the drug of choice for some human microsporidial infections. Results vary from organism clearance (*Encephalitozoon*) to resistance (*Enterocytozoon*). This study investigated albendazole efficacy on *A. algerae* infection in rabbit kidney (RK13) cells and human fetal lung (HFL-1) fibroblasts. This drug is effective on *A. algerae*, and the 50% inhibitory concentration in RK13 cells is 0.1 μg/ml. Long-term therapy can inhibit 98% spore production; however, if the treatment is interrupted, the infection is reestablished without documented additional patient exposures. The *A. algerae* beta-tubulin gene was purified, cloned, and then sequenced. Specific amino acids linked to benzimidazole sensitivity (five out of six) are conserved in *A. algerae*. **Although these data suggest that *A. algerae* is sensitive to albendazole,** organisms in culture are not completely eliminated.

Hocevar SN, Paddock CD, Spak CW, Rosenblatt R, Diaz-Luna H, Castillo I, Luna S, Friedman GC, Antony S, Stoddard RA, Tiller RV, Peterson T, Blau DM, Sriram RR, da Silva A, de Almeida M, Benedict T, Goldsmith CS, Zaki SR, Visvesvara GS, Kuehnert MJ, Microsporidia Transplant Transmission Investigation Team. 2014. Microsporidiosis acquired through solid organ transplantation: a public health investigation. *Ann Intern Med* **160:**213–220.

Encephalitozoon cuniculi **has been associated with renal, respiratory, and central nervous system infections in immunosuppressed patients. This microsporidian has also been identified as causing illness among three recipients of solid organ transplants from a single donor.** Appropriate specimens were examined via culture, immunofluorescent antibody, PCR, immunohistochemistry, and electron microscopy. Medical records from the donor were reviewed, as were answers from a clinically relevant questionnaire. Kidneys and lungs from the donor were transplanted to three recipients. Subsequently, the recipients became symptomatic with fever approximately 7 to 10 weeks posttransplantation. Urine cultures, serologic results, and PCR testing from the three recipients were all positive for *E. cuniculi,* genotype III. *E. cuniculi* was also confirmed in all biopsy and autopsy specimens. Although autopsy specimens were not available, serologic test results for *E. cuniculi* were also found from the donor. The recipients were treated with albendazole. Assessment of the donor information failed to confirm specific factors related to the suspected *E. cuniculi* infection. Microsporidiosis has now been confirmed as an emerging transplant-associated disease and should be considered in symptomatic transplant recipients. **Possible donor-derived disease assessment is critical, especially when multiple recipients from a common donor become symptomatic.**

Watts MR, Chan RC, Cheong EY, Brammah S, Clezy KR, Tong C, Marriott D, Webb CE, Chacko B, Tobias V, Outhred AC, Field AS, Prowse MV, Bertouch JV, Stark D, Reddel SW. 2014. *Anncaliia algerae* microsporidial myositis. *Emerg Infect Dis* **20:**185–191.

The microsporidian *Anncaliia algerae* was documented in 2004 as causing fatal myositis in an immunosuppressed patient from Pennsylvania. Two cases were later reported, and the authors reveal two additional cases, including one nonfatal case. All five cases were investigated, including clinical findings, diagnosis, and management; life cycle and epidemiology information was also summarized. **Prior to infection, all patients were immunosuppressed due to relevant therapy for rheumatoid arthritis or solid organ transplantation.** Four of the five patients were from Australia. The diagnosis was confirmed from skeletal muscle biopsies. The surviving patient was treated with albendazole and had immunosuppressive drugs reduced. While insects serve as the normal hosts for *A. algerae*, it has been suggested that human contact with water contaminated by *A. algerae* spores may lead to infections. **Therefore, *A. algerae* has been documented as causing human myositis, particularly in coastal areas in Australia.**

Cystoisospora (*Isospora*)

Lindsay DS, Houk AE, Mitchel SM, Dubey JP. 2014. Developmental biology of *Cystoisospora* (*Apicomplexa*: *Sarcocystidae*) monozoic tissue cysts. *J Parasitol* **100:**392–398.

Tissue cyst stages form an integral part of the developmental cycle and potential transmission of *Sarcocystidae* species. Tissue cysts within these species (*Toxoplasma*, *Hammondia*, *Neospora*, *Besnoitia*, and *Sarcocysti*) contain many infectious stages (bradyzoites). The tissue cyst stage of *Cystoisospora* (syn. *Isospora*) has one infectious stage (zoite) and is therefore referred to as a monozoic tissue cyst (MZTC). No tissue cyst stages are known for the *Nephroisospora*. The current report looks at the developmental biology of MZTC stages of *Cystoisospora* Frenkel, 1977, the cause of intestinal coccidiosis in cats, dogs, pigs, and humans. **These MZTC stages of *Cystoisospora belli* are possibly associated with human clinical disease reoccurrence.** Developmental stages that are found in low numbers in immunocompetent definitive human hosts tend to be much more numerous in immunosuppressed humans. It is anticipated that additional studies will lead to a clarification of *C. belli* infections in immunocompromised patients, as well as any life cycle mechanisms associated with possible reoccurrence of disease.

Sarcocystis spp.

Lingappa HA, Krishnamurthy A, Puttaveerachary AK, Govindashetty AM, Sahni S. 2015. Foray of cytologically diagnosed intramuscular sarcocystosis—a rarity. *J Clin Diagn Res* **9:**ED11–ED12.

While human cases with *Sarcocystis* spp. are uncommon, these organisms are well documented to cause intestinal and muscular problems. In this particular patient, an intramuscular swelling in the lumbar region was diagnosed through cytology as intramuscular sarcocystosis; this finding was later confirmed from histopathology. This case emphasizes the importance of fine needle aspiration cytology in differentiating sarcocystosis from other intramuscular parasites. **In this case, the overall parasite morphology was more definitive from the cytology smears than the traditional paraffin sections and strongly suggests the benefits of using fine needle aspirate cytology.** There are excellent images in this particular article.

Fayer R, Esposito DH, Dubey JP. 2015. Human infections with *Sarcocystis* species. *Clin Microbiol Rev* **28:**295–311.

Recurrent outbreaks of human cases of muscular sarcocystosis in travelers to Malaysia have led to increased interest in this disease. The *Sarcocystis* life cycle requires two hosts, definitive and intermediate; humans can serve as definitive hosts with intestinal sarcocystosis (*Sarcocystis hominis* from beef, and *Sarcocystis suihominis* from pork). Humans serve as intermediate hosts for *Sarcocystis nesbitti* (reptile definitive host, and possibly other unidentified species) acquired by ingesting sporocysts from feces-contaminated food or water and the environment. **It is important to consider this infection in patients with a relevant travel history to tropical areas of the world, particular if they present with elevated serum enzyme levels and eosinophilia.**

Protozoa from Other Body Sites

Free-Living Amebae

Robaei D, Carnt N, Minassian DC, Dart JK. 2014. The impact of topical corticosteroid use before diagnosis on the outcome of *Acanthamoeba* keratitis. *Ophthalmology* **121:**1383–1388.

Corticosteroid use prior to the diagnosis of *Acanthamoeba* keratitis (AK) is strongly linked to a worse visual outcome. This situation often occurs due to the initial misdiagnosis of AK as herpetic keratitis. It is clinically critical to include AK in the differential diagnosis of keratitis causative agents, particularly before diagnosing herpes keratitis and instituting the subsequent use of topical corticosteroids.

Van der Beek NA, van Tienen C, de Haan JE, Roelfsema J, Wismans PJ, van Genderen PJJ, Tanghe HL, Verdijk RM, Titulaer MJ, van Hellemond JJ. 2015. Fatal *Balamuthia mandrillaris* meningoencephalitis in the Netherlands after travel to The Gambia. *Emerg Infect Dis* **21:**896–898.

Up to this point, meningoencephalitis caused by *Balamuthia mandrillaris* had not been reported from Africa. Unlike *Acanthamoeba* encephalitis, *Balamuthia* amebic encephalitis has been found in immunocompetent individuals. These findings confirm the virulence of this pathogen. In immunocompetent hosts, an inflammatory response is mounted and amebae are surrounded by macrophages, lymphocytes, and neutrophils. However, with rare exceptions, these patients also tend to die of severe central nervous system disease. Findings are often nonspecific, including an elevated cerebrospinal fluid protein and low cerebrospinal fluid glucose. **Infection with this organism should be considered in unexplained cases of meningoencephalitis, including those in immunocompetent patients.** In areas of the world where reports are lacking or are minimal, the infection may go unrecognized.

Trichomonas vaginalis

Hathorn E, Ng A, Page M, Hodson J, Gaydos C, Ross JD. 2014. A service evaluation of the Gen-Probe APTIMA nucleic acid amplification test for *Trichomonas vaginalis*: should it change whom we screen for infection? *Sex Transm Infect* **91:**81–86.

This study involves evaluation of the Gen-Probe APTIMA nucleic acid amplification test used to determine the prevalence of *Trichomonas vaginalis* infection in a United Kingdom sexual health clinic. Also, a part of the study was designed to identify possible risk factors to develop an appropriate patient screening strategy. Patients (new clinical episode) were offered the Aptima test as well as routine screening. Asymptomatic females submitted a self-collected vulvovaginal specimen, and asymptomatic men submitted a first-void urine sample. A urethral swab was taken from symptomatic men, and two posterior vaginal swabs from symptomatic females (Aptima and culture). Data were collected from all patients who were positive for *T. vaginalis* infection, as well as 100 randomly selected *T. vaginalis*–negative controls. The prevalence of *T. vaginalis* infection was 21/1,483 (1.4%) (95% confidence interval [CI], 0.9% to 2.2%) in men and 72/2,020 (3.6%) (95% CI, 2.8% to 4.5%) in women. The *T. vaginalis* positivity rate was higher in black Caribbean patients when compared with Caucasian patients (men 5.4% versus 0.1%, $P < 0.001$; women 9.0% versus 1.2%, $P < 0.001$). Compared to culture results, the Aptima test detected 16 more infections (38%) in symptomatic women. **Although more inclusive screening with the Aptima test will identify additional *T. vaginalis* infections, prevalence in the UK is low, and the extra screening may not prove to be cost-effective. However, testing of symptomatic patients and those within high-risk asymptomatic groups may be clinically warranted.**

Nathan B, Appiah J, Saunders P, Heron D, Nichols T, Brum R, Alexander S, Baraitser P, Ison C. 2014. Microscopy out performed in a comparison of five methods for detecting *Trichomonas vaginalis* in symptomatic women. *Int J STD AIDS* **26:**251–256.

This study was designed to compare five methods (an in-house PCR, the Aptima *T. vaginalis* kit, the OSOM Trichomonas Rapid Test, culture, and microscopy) to detect *Trichomonas vaginalis* infection in symptomatic women. A true positive was defined as any sample that was positive for *T. vaginalis* by two or more methods. Of 246 women, 24 were found positive for *T. vaginalis* by two or more different methods. From this group of 24 patients, 21 were real-time PCR positive (sensitivity 88%), 22 were Aptima *T. vaginalis* positive (sensitivity 92%), 22 were OSOM positive (sensitivity 92%), 9 were wet mount microscopy positive (sensitivity 38%), and 21 were culture positive (sensitivity 88%). Two patients were not considered true positives, because they were positive using one method only. **All methods demonstrated a sensitivity that was significantly greater than that obtained using wet mount microscopy. Thus, many symptomatic cases are routinely missed if microscopy is the only method used for the diagnosis of trichomoniasis.**

Secor WE, Meites E, Starr MC, Workowski KA. 2014. Neglected parasitic infections in the United States: trichomoniasis. *Am J Trop Med Hyg* **90:**800–804.

Trichomonas vaginalis is the most prevalent nonviral sexually transmitted infection within the United States. However, the problems associated with this infection are not thoroughly understood, and the infection receives too little attention regarding public health issues. Serious infection sequelae include an increased risk of infection with HIV and unfavorable pregnancy outcomes such as premature rupture of the membranes, preterm labor, and low-birth-weight infants. **Newly developed diagnostic tests have improved the diagnosis, but infections in males require additional study and clarification.** Other issues that need additional investigation include the epidemiology of trichomoniasis in both sexes, defining the actual public health impact of symptomatic and asymptomatic *T. vaginalis* infections, and therapeutics and their efficacy in reducing the overall costs of this disease. **Other important issues for clarification include resistance versus noncompliance with therapy and the probability of reinfection.**

Tissue Protozoa

Toxoplasma gondii

McAuley JB. 2014. Congenital toxoplasmosis. *J Pediatric Infect Dis Soc* **3:**S30–S35.

Infection with *Toxoplasma gondii* is a very common human parasitic infection; however, patients generally remain asymptomatic. Serious sequelae in a pregnant woman with a primary infection, particularly early in the pregnancy, can cause very serious disease in the developing fetus. These potential problems, including organism potential infectivity and severity of symptoms, have recently been linked to various *Toxoplasma* genotypes. Risk factors, including foodborne transmission, have been more thoroughly investigated and defined. Early diagnosis in infected pregnant women, followed by recommended therapy, has decreased mother-to-child transmission. The addition of immunoglobulin G avidity testing has also produced improved diagnostic testing and better clinical outcomes during pregnancy. It is now well documented that the infected child can be treated *in utero* and through the first year of life. **However, regardless of our more complete comprehension of congenital disease, other issues remain problematic and require clarification. These include the development of enhanced diagnostic tests, the need for more effective therapy, clearly defined screening protocols for pregnant women, and food safety recommendations for disease prevention.**

Davis SM, Anderson BL, Schulkin J, Jones K, Eng JV, Jones JL. 2014. Survey of obstetrician-gynecologists in the United States about toxoplasmosis: 2012 update. *Arch Gynecol Obstet* **291:**545–555.

The American College of Obstetricians and Gynecologists (ACOG) surveyed a number of their members to establish current knowledge, practices, opinions, and educational preferences regarding toxoplasmosis during pregnancy. Among the respondents, 80.2% had reported no acute maternal *Toxoplasma gondii* infections during the past 5 years, 12.7% correctly identified the clinical relevance of the *Toxoplasma* avidity test for screening, and 42.6% performed *T. gondii* serologies for some asymptomatic pregnant women. Respondents in the northeastern United States were twice as likely to use routine screening when compared with those in the west; female physicians were one and a half times more likely than male physicians to use routine screening. Most practitioners felt that updated ACOG guidelines on screening and management for pregnant women with acute toxoplasmosis would support educational efforts related to this infection. Additional information related to risk factor counseling would also be helpful.

Plasmodium and *Babesia* spp.

Plasmodium spp.

Vythilingam I, Lim YA, Venugopalan B, Ngui R, Leong CS, Wong ML, Khaw L, Goh X, Yap N, Sulaiman WY, Jeffery J, Zawiah AG, Nor Aszlina I, Sharma RS, Yee Ling L, Mahmud R. 2014. *Plasmodium knowlesi* malaria an emerging public health problem in Hulu Selangor, Selangor, Malaysia (2009-2013): epidemiologic and entomologic analysis. *Parasit Vectors* **7:**436.

A review of 100 *Plasmodium knowlesi*/*Plasmodium malariae* cases over a 5-year period in the Hulu Selangor district found these cases to be predominantly seen among young adults (ages 20 to 39 years; 67 cases; 67%); no deaths were reported. In most cases, the individuals worked in agriculture and forestry (51; 51%). Information based on the examination of 535 mosquitoes indicated *Anopheles maculatus* was the predominant species (49.5%), followed by *Anopheles letifer* (13.1%) and *Anopheles introlatus* (11.6%). Only *A. introlatus* was positive for *Plasmodium* oocysts. The authors demonstrated that *P. knowlesi* hotspot areas overlapped with areas where the infected *A. introlatus* was found, thus strongly supporting the hypothesis that *A. introlatus* is the vector for *P. knowlesi* in the Hulu Selangor district. Unfortunately, without additional information on the vectors and macaques, effective control measures will be difficult to implement. The use of a GIS (geographic information system) will be useful in defining these hotspot areas for control implementation. **Therefore, *A. introlatus* has been implicated as a vector of *P. knowlesi* in Hulu Selangor. These cases of *P. knowlesi* continue to increase.**

Nakaviroj S, Kobasa T, Teeranaipong P, Putaporntip C, Jongwutiwes S. 2015. An autochthonous case of severe *Plasmodium knowlesi* malaria in Thailand. *Am J Trop Med* **92:**569–572.

The patient was a 58-year-old man who presented with fever, hypotension, renal failure, jaundice, and severe thrombocytopenia. The patient's parasitemia on admission was 16.67%, a very high percentage. *Plasmodium knowlesi* is unique among the primate and human malarias in that it has a 24-h erythrocytic cycle, which tends to accelerate the development of complications. Since *P. knowlesi* malaria can progress rapidly to severe disease, these cases should be treated like *Plasmodium falciparum* malaria if the species identification is based on microscopic examination alone or if coinfection with *P. falciparum* cannot be excluded with certainty using PCR. **It is important to remember that diagnostic results should include a comment: "Unable to rule out *P. falciparum* or *P. knowlesi*." This information alerts the physician to the possibility of severe disease and possible fatal outcomes.**

Maude RJ, Nguon C, Dondorp AM, White LJ, White NJ. 2014. The diminishing returns of atovaquone-proguanil for elimination of *Plasmodium falciparum* malaria: modelling mass drug administration and treatment. *Malar J* **13:**380.

Artemisinin resistance has been identified as a major problem in the elimination of *Plasmodium falciparum* malaria, which depends on the use of artemisinin combination therapy (ACT). In areas where artemisinin-resistant *P. falciparum* occurs, atovaquone-proguanil (A-P) has been recommended as an alternative to ACT. However, a single point mutation in the cytochrome *b* gene is responsible for resistance to atovaquone. Even a single round of mass drug administration (MDA) with A-P could lead to resistance. Within 4 to 5 years, the use of A-P for symptomatic patients could result in complete therapeutic failure. **Therefore, to achieve malaria elimination, A-P for MDA or treatment of symptomatic patients should be discouraged. A combination strategy using ACT for treatment of symptomatic patients, insecticide-impregnated bed nets, and ACT+P MDA is recommended.**

Kobylinski KC, Alout H, Foy BD, Clements A, Adisakwattana P, Swierczewski BE, Richardson JH. 2014. Rationale for the coadministration of albendazole and ivermectin to humans for malaria parasite transmission control. *Am J Trop Med Hyg* **91:**655–662.

It has been established that infections with soil-transmitted helminths (STHs) increase the risk for malaria; STHs may also increase the efficacy of malaria transmission. With the potential for developing more pronounced anemia in patients who have both infections and the potential for pregnancy outcome complications, it has been suggested that the **addition of ivermectin mass drug administration added to the administration of albendazole would serve as a potential malaria control mechanism through elimination of STHs.**

Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. 2014. Global extent of chloroquine-resistant *Plasmodium vivax*: a systematic review and meta-analysis. *Lancet Infect Dis* **14:**982–991.

In most areas of endemicity, chloroquine remains the first-line drug for *Plasmodium vivax* malaria; however, resistance continues to increase. In infections with *P. vivax*, determination of therapeutic success is complicated by a potential relapse from the dormant liver stages. The authors identified 129 eligible clinical trials involving 21,694 patients at 179 study sites and 26 case reports describing 54 patients. They identified chloroquine-resistant areas in 58 (53%) of 113 study sites, which were seen in most countries where *P. vivax* was endemic. Organism clearance in 95% of patients by day 2 or 100% of patients by day 3 was correct in predicting chloroquine sensitivity. Unfortunately, varying study designs and data analysis have been somewhat confusing in the surveillance of chloroquine-resistant *P. vivax*. Improved methods for tracking chloroquine resistance in these areas are necessary for the development of improved malaria therapeutics.

Gonçalves LA, Cravo P, Ferreira MU. 2014. Emerging *Plasmodium vivax* resistance to chloroquine in South America: an overview. *Mem Inst Oswaldo Cruz* **109:**534–539.

The global emergence of *Plasmodium vivax* strains resistant to chloroquine definitely impacts current international approaches to malaria control and eradication. **These strains are highly endemic in Indonesia, East Timor, and Papua New Guinea. In addition, chloroquine-resistant *P. vivax* infections have also been confirmed in Guyana, Peru, and Brazil.** The authors discuss chloroquine resistance throughout areas of *P. vivax* endemicity in Latin America. They also include the molecular mechanisms of chloroquine resistance in *P. vivax*, as well as the potential development of dependable molecular drug resistance markers that can predict therapeutic outcomes.

Khim N, Andrianaranjaka V, Popovici J, Kim S, Ratsimbasoa A, Benedet C, Barnadas C, Durand R, Thellier M, Legrand E, Musset L, Menegon M, Severini C, Nour BY, Tichit M, Bouchier C, Mercereau-Puijalon O, Menard D. 2014. Effects of mefloquine use on *Plasmodium vivax* multidrug resistance. *Emerg Infect Dis* **20:**1629–1636.

Although drug resistance is well documented in *Plasmodium falciparum*, the inability to culture *Plasmodium vivax* limits our understanding of drug resistance in this species. In areas where mefloquine has been used as a monotherapy or in combination with artemisinin, mefloquine resistance has been detected. To determine the impact of chloroquine resistance on *P. vivax*, the authors used PCR for the assessment of *P. vivax mdr-1* gene copies for the evaluation of this gene as an indicator of *P. vivax* in patients documented to have received chloroquine. Currently, mefloquine has been used for chemoprophylaxis in over 35 million travelers from France, alone. *P. falciparum mdr-1* gene amplification is the primary mechanism of mefloquine resistance in this species; in areas where mefloquine has been widely used, *P. vivax mdr-1* amplification is frequent and detected in high numbers of isolates. **Similar to results for atovaquone resistance, data from this study suggest that *PVmdr-1* amplification is probably faster than previously thought, thus possibly leading to an increase of mefloquine resistance in *P. vivax*.**

Jaurequiberry S, Ndour PA, Roussel C, Ader F, Safeukui I, Nguyen M, Biliqui S, Ciceron L, Mouri O, Kendjo E, Bricaire F, Vray M, Angoulvant A, Mayaux J, Haldar K, Mazier D, Danis M, Caumes E, Thellier M, Buffet P, French Artesunate Working Group. 2014. Postartesunate delayed hemolysis is a predictable event related to the lifesaving effect of artemisinins. *Blood* **124:**167–175.

It has been well documented that patients with severe malaria treated with artesunate may experience delayed hemolysis (1 to 3 weeks post-therapy) with pitting, a splenic process during which dead organisms are removed from red blood cells. These patients are often nonimmune travelers rather than patients who live in areas of endemicity. The authors monitored 123 patients treated for severe malaria with artesunate. Thirteen of sixty (22%) nontransfused patients had delayed hemolysis. **Health care workers need to be aware of potential complications, which strongly suggest extended patient follow-up for potential hemolytic problems associated with the use of artesunate.**

Fuehrer HP, Noedl H. 2014. Recent advances in detection of *Plasmodium ovale*: implications of separation into the two species *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi*. *J Clin Microbiol* **52:**387–391.

Plasmodium ovale is endemic in most countries where malaria is endemic in southern and southeastern Asia, and both classic (*P. ovale curtisi*) and variant (*P. ovale wallikeri*) types have been seen. Molecular phylogenetic data indicate that *P. ovale* malaria is caused by two closely related species. The authors review various methods for the molecular diagnosis of malaria, emphasizing the detection of *P. ovale wallikeri* and *P. ovale curtisi*. Both species occur in Africa, as well; dual infections with both organisms have been documented. Although the two species cannot be differentiated on the basis of morphology, they have been reported to vary in terms of latency. An update for the widely used NP-1993 to NP-2005 (SSU rRNA) protocols for all human malaria parasites is discussed, specifically discussing the two primers (rOVA1WC and rOVA2WC) that bind to the two *P. ovale* species but do not bind to other human malaria parasites. **If two different PCRs for *P. ovale curtisi* and *P. ovale wallikeri* are inaccessible, the *P. ovale* sp. should be identified using techniques designed to detect both species. In this case, a more definitive answer should be obtained if possible as follow-up testing.**

Ta TH, Hisam S, Lanza M, Jiram AI, Ismail N, Rubio JM. 2014. First case of a naturally acquired human infection with *Plasmodium cynomolgi*. *Malar J* **13:**68–75.

Since 1960, seven species of simian malaria have been confirmed to be transmissible from mosquito to human: *Plasmodium cynomolgi*, *Plasmodium brasilianum*, *Plasmodium eylesi*, *Plasmodium knowlesi*, *Plasmodium inui*, *Plasmodium schwetzi*, and *Plasmodium simium*. However, *P. knowlesi* is the only one that infects humans in nature. **The authors report the first known case of a human naturally acquired *P. cynomolgi* malaria infection.** The patient was a 39-year-old female from a nonendemic area, had no history of malaria, and reported no travel to possible endemic areas. Stained thick and thin blood films revealed *P. malariae/P. knowlesi*. However, the diagnosis of *P. vivax* was confirmed by a nested PCR assay. Using another PCR assay plus sequencing confirmed that the patient was infected with *P. cynomolgi* and not with *P. vivax*. This is the first report of human *P. cynomolgi* infection acquired via mosquito bite. However, other similar cases may remain undiagnosed or misdiagnosed, since *P. cynomolgi* cannot be differentiated morphologically from *P. vivax*. Also, depending on the molecular test used, the diagnosis could also be *P. vivax* in error.

Muller M, Schlagenhauf P. 2014. *Plasmodium knowlesi* in travellers: update 2014. *Int J Inf Dis* **22:**55–64.

This infection has taken on added importance for travelers; *Plasmodium knowlesi* imported infections have been reported in many areas worldwide outside of the documented areas of endemicity. From a detailed study of 103 publications, the following facts were found: the time spent in the endemic areas was from 1 week to several months and included travel to a forested area with overnight stays. These travelers were from Sweden, Finland, Spain, France, the Netherlands, Germany, Australia, New Zealand, and the United States; most patients were males. While fever and chills were reported in 100% of the patients, headache, rigors, malaise, anorexia, myalgia, arthralgia, cough, abdominal pain, tachycardia, and tachypnea were also reported. Although thick and thin blood films remain the tests of choice, there can be confusion since *P. knowlesi* resembles *P. falciparum* in the early red blood cell stages and *P. malariae* in the later red blood cell stages. Unfortunately, the rapid malaria tests have limited sensitivity in distinguishing nonfalciparum species. **This review covers all aspects of *P. knowlesi* malaria in travelers and will help clarify this infection and its sequelae.**

Moreno A, Joyner C. 2015. Malaria vaccine clinical trials: what's on the horizon. *Curr Opin Immunol* **35:**98–106.

Although significant progress has been made regarding potential malaria vaccines, much work remains. This review contains extensive information on completed and ongoing vaccine trials, as well as information on newly discovered vaccine candidates.

Babesia spp.

Apostolou A, Sorhage F, Tan C. 2014. Babesiosis surveillance, New Jersey, USA, 2006–2011. *Emerg Infect Dis* **20:**1407–1409.

During 2006 to 2011, 568 babesiosis cases were reported in New Jersey: 521 (92%) confirmed and 47 (8%) probable. In 2006 and 2011, 64 (0.76/100,000) and 166 (1.97/100,000) cases were reported, a figure that represents a 260% increase. Seven counties accounted for 462 (81%) of the reported cases, and 128/166 (77%) reported cases occurred in 2011. While all 21 counties reported at least 1 case in 2006 to 2011, only 8 counties reported any cases from 1993 to 2001. Counties in southern New Jersey reported most of the babesiosis cases; these same counties also reported a higher prevalence of Lyme disease. It has been well documented that the relevant ticks can carry and transmit both infections at the same time. Although babesiosis is found in both eastern and western areas within the United States, expanded surveillance throughout the whole area is recommended, particularly when related to potential donor transfusion cases. The majority of babesiosis cases in this report were seen during the summer; however, documentation of transfusion cases was confirmed during the entire year. Adding babesiosis to the list of reportable diseases will enhance current reporting practices and will support efforts to redefine the overall epidemiology of this disease.

Schmidt M, Geilenkeuser WJ, Sireis W, Seifried E, Hourfar K. 2014. Emerging pathogens: how safe is blood? *Transfus Med Hemother* **41:**10–17.

For many years, the issue of donated blood safety was primarily focused on the possible transmission of HIV. However, if considering other potential transfusion-related infections, malaria, dengue virus, *Trypanosoma cruzi* (Chagas' disease), and *Babesia* spp. also need to be included. More than 300 million to 500 million individuals throughout the world are infected with malaria, and 1.5 million to 2.7 million people a year, half of whom are children under the age of 5, die from the disease. Dengue virus, which causes hemorrhagic fever and shock syndrome, infects more than 50 million people each year. Chagas' disease caused by *Trypanosoma cruzi* occurs primarily in South America and infects roughly 10 million people each year; this infection is now endemic in North America, both as a natural infection and related to immigration into the United States and Canada.

Babesia spp. cause a parasitic infection that resembles malaria infection. While patients with babesiosis usually remain asymptomatic, severe disease has been reported in those who may be immunocompromised, including the elderly and very young. The use of detection systems for all of these organisms related to donor screening becomes very costly and time consuming. Another approach recommends organism inactivation in donor blood, which may provide a comprehensive solution for potential blood-borne pathogens. This approach will also decrease the potential loss of blood donors through the deferral process.

Leishmania spp.

Castelli G, Galante A, Lo Verde V, Migliazzo A, Reale S, Lupo T, Piazza M, Vitale F, Bruno F. 2014. Evaluation of two modified culture media for *Leishmania infantum* cultivation versus different culture media. *J Parasitol* **100:**228–230.

This publication reports on the use of improved culture media for *Leishmania* promastigotes that eliminates the need for Evans' modified Tobie's medium (EMTM), liquid RPMI 1640, and Peptone-yeast extract medium (P-Y). Although EMTM supports parasite growth very well, it is complicated to prepare and requires fresh rabbit blood. RPMI 1640 and P-Y do not support parasite growth as well as EMTM. The authors provide information on new media modifications, called RPMI-PY and Tobie-PY for the growth of *Leishmania*. **Based on comparisons of the classic culture media to the new media, RPMI-PY and Tobie-PY, results indicate that the new media provide faster and more abundant growth.**

Saroufim M, Charafeddine K, Issa G, Khalifeh H, Habib RH, Berry A, Ghosn N, Rady A, Khalifeh I. 2014. Ongoing epidemic of cutaneous leishmaniasis among Syrian refugees, Lebanon. *Emerg Infect Dis* **20:**1712–1715.

The authors have documented a cutaneous leishmaniasis outbreak that began among Syrian refugees in Lebanon in September of 2012. Patients (1,275) from 213 refugee families were placed into three groups: those in whom leishmaniasis was diagnosed, confirmed, and treated; those in whom leishmaniasis was diagnosed, confirmed, and not treated; and those in whom leishmaniasis was not diagnosed. For 948 patients from the undiagnosed group, clinical specimens were obtained using punch biopsies for microscopic examination and molecular confirmation. The authors identified *Leishmania tropica* in 85%, a species causing large facial lesions and prolonged disease requiring systemic rather than localized therapy, and *Leishmania major* in 15% of patients. **After 3 months of megulamine antimonate therapy, the cure rate was 82%, with 80% of the patients receiving intralesional therapy and 20% receiving intramuscular therapy.** Risk factors for cutaneous leishmaniasis include many conditions facing refugee populations, including poverty, malnutrition, inadequate sanitation, underlying illness, and inadequate housing. Unfortunately these conditions support the sandfly vectors of *L. tropica*, and leishmaniasis becomes a tremendous problem for these populations.

Glazer Y, Gandacu D, Anis E, Karakis I, Warshavsky B, Slater P, Grotto I. 2014. Resurgence of cutaneous leishmaniasis in Israel, 2001–2012. *Emerg Infect Dis* **20:**1605–1617.

Cutaneous leishmaniasis has been endemic in Israel for many years, and the disease is known as the "Rose of Jericho." **However, after a relatively stable incidence rate of 0.4/100,000 population in 2001, the rate increased in 2012 to 4.4/100,000 population. Also, the disease became more numerous in areas with limited previous reports.** Based on an extensive epidemiologic analysis of the cases submitted, the authors reported an expansion of *Leishmania major* infections throughout the southern part of Israel and more concentrated outbreaks with *Leishmania tropica* in the northern part of Israel. Increased incidence of *L. tropica* has also been reported from other countries in the Mediterranean Basin. Increases in cutaneous leishmaniasis are often linked to migration, urbanization, deforestation, climate change, and improved disease diagnosis. Certainly, changes in the population dynamics of the sand fly vectors probably play a large role in disease epidemiology.

Martin-Blondel G, Iriart X, El Baidouri F, Simon S, Mills D, Demar M, Pistone T, Le Taillandier T, Malvy D, Gangneus J, Couppie P, Munckhof W, Marchou B, Ravel C, Berry A. 2015. Outbreak of *Leishmania braziliensis* cutaneous leishmaniasis, Saül, French Guiana. *Emerg Inf Dis* **21:**892–894.

A documented increase and geographic expansion of *Leishmania braziliensis* in French Guiana are associated with the need for species-specific identification of the causative agent. This organism is responsible for involving the mucous membranes of the lips, nose, soft palate, and larynx. Unfortunately, *L. braziliensis* is refractive to pentamidine isethionate, which is the primary drug used for *Leishmania guyanensis* in the same geographic area. **Clinical cases of cutaneous leishmaniasis from this region require careful diagnosis and appropriate therapy, both of which are subject to error.**

Trypanosoma spp.

African Trypanosomiasis

Franco JR, Simarro PP, Diarra A, Jannin JG. 2014. Epidemiology of human African trypanosomiasis. *Clin Epidemiol* **6:**257–275.

Human African trypanosomiasis (HAT) is caused by *Trypanosoma brucei gambiense*, which is a chronic form of the disease present in western and central Africa (including actual sleeping sickness syndrome), and by *Trypanosoma brucei rhodesiense*, which is an acute disease located in eastern and southern Africa (much higher morbidity than the western form). While the eastern form has many game animals involved as reservoirs, the western form is characterized by humans being the main reservoir. In the gambiense form 98% of the cases are reported, with 75% of the cases coming from the Democratic Republic of the Congo. This infection is seen in defined rural areas that are located in sub-Saharan Africa. The risk of infection is influenced by the interaction of the infected tsetse fly with the potential human host. **After a series of outbreaks and disease resurgence and control, elimination of the gambiense form of HAT is thought to be a possibility by 2030. However, since the rhodesiense form of HAT is a zoonosis involving many animal reservoirs, control of this infection is much less likely, even with more concentrated control efforts over time.**

American Trypanosomiasis

Andrade DV, Gollob KJ, Dutra WO. 2014. Acute Chagas' disease: new global challenges for an old neglected disease. *PLoS Negl Trop Dis* **8:**e3010. doi:10.1371/journal.pntd.0003010.

Chagas' disease is caused by the bloodborne protozoan *Trypanosoma cruzi*, with approximately 7 to 8 million cases worldwide. This disease continues to expand its geographic range and remains a serious global public health problem. Although a great deal is known about the chronic phase of human Chagas' disease, much less is well defined regarding the acute phase of the disease. In this review, the authors discuss (i) the current status of acute Chagas' disease worldwide, (ii) the patient immune response and its impact on disease outcomes, and (iii) the **reactivation of disease in immunocompromised individuals (particularly relevant for organ transplantation recipients and the treatment of patients with HIV infection).** This review includes updates on oral infection (contaminated fruit and fruit juices), congenital infections, blood transfusions and solid organ transplantation, potential problems with diagnosis of acute infections, immune responses in the murine model, the importance of cytokines, and reactivation of disease in immunocompromised patients.

Tenney TD, Curtis-Robles R, Snowden KF, Hamer SA. 2014. Shelter dogs as sentinels for *Trypanosoma cruzi* transmission across Texas. *Emerg Infect Dis* **20:**1323–1326.

Chagas' disease, an infection caused by *Trypanosoma cruzi*, has recently been diagnosed in humans in the southern United States. This infection is now endemic in a number of the southern states. The vectors are also found in many areas throughout the United States. **The authors evaluated exposure of shelter dogs in Texas to *T. cruzi*. They also determined that seroprevalence data for a number of different regions was 8.8%,** a figure that may actually be a low estimate. Serosurveillance of various canine populations has become a helpful mechanism for assessing public health risks associated with this infection. **It is important for health care workers to become more aware of the vectors (kissing bugs, triatomid bugs) of *T. cruzi* and their ecologic niches, as well as the potential risks associated with Chagas' disease in areas where seropositive dogs have been confirmed. Also, it is unknown whether dogs serve as reservoir hosts for *T. cruzi* in the United States; certainly, this has been confirmed from some areas in Central and South America.**

Kransdorf EP, Zakowski PC, Kobashigawa JA. 2014. Chagas disease in solid organ and heart transplantation. *Curr Opin Infect Dis* **27:**418–424.

Diagnosis and management of infections with *Trypanosoma cruzi*, the cause of Chagas' disease, have become much more important in solid organ transplantation patients. This is particularly true in cardiac transplant patients who are at much greater risk of complications than with liver or kidney transplantation. Each year, approximately 2% of infected individuals will progress to Chagas cardiomyopathy. For these transplant patients, comprehensive monitoring for possible disease reactivation is critical for a good clinical outcome. Molecular testing of peripheral blood is more sensitive and much more likely to detect a reactivation process before symptoms are evident. **Transplant teams should implement thorough surveillance protocols for the detection of disease reactivation in both donors and recipients. Although Chagas' disease may seem unusual and infrequent, it is becoming more prevalent in many parts of the world, including the United States.**

Garza M, Feria Arroyo TP, Casillas EA, Sanchez-Cordero V, Rivaldi CL, Sarkar S. 2014. Projected future distributions of vectors of *Trypanosoma cruzi* in North America under climate change scenarios. *PLoS Negl Trop Dis* **8:**e2818. doi:10.1371/journal.pntd.0002818.

Trypanosoma cruzi is transmitted by insects of the family *Reduviidae*, subfamily *Triatominae* (kissing bugs, triatomid bugs). The infection is known as Chagas' disease, which kills around 45 thousand people each year and impacts 10 million people in Latin America and the southern United States. It has been estimated that 300,000 infected people are present throughout Canada and the United States. Epidemiologists predict climate change will influence overall vector distribution, which in turn will alter the overall potential risk of human Chagas' disease. **It has been well documented that vector habitat in the United States continues to expand northward. The authors predict a continued northern shift in the territory of *Trypanosoma gerstaeckeri* and a northern and southern territory shift of *Trypanosoma sanguisuga* due to climate change.**

Martin DL, Goodhew B, Czaicki N, Foster K, Rajbhandary S, Hunter S, Brubaker SA. 2014. *Trypanosoma cruzi* survival following cold storage: possible implications for tissue banking. *PLoS One* **9:**e95398. doi:10.1371/journal.pone.0095398.

Trypanosoma cruzi, the agent of Chagas' disease, can be transmitted via insect vectors, solid organ transplantation, or transfusion of contaminated blood or blood products. In transplantation- or transfusion-related cases, transmission of the causative agent depends on storage conditions used to hold human tissues. The authors report on a number of storage parameters that were not successful in killing the organisms. Trypomastigotes survived and remained infective 24 hours in blood held at room temperature; 24 and 48 hours when held at 4°C; in 2/13 cultures held 28 days at 4°C; and in all specimens stored 365 days at −80°C without cryoprotectant. **If one accepts the reported rate of *T. cruzi* infection in U.S. blood donors of 1:27,000, with approximately 30,000 tissue donors each year, it is highly likely that one or two positive tissue donors will be detected.** While a number of techniques are used for blood product and tissue allograft protection from pathogens, much more work is required to clarify parasite survival and potential risk factors related to *T. cruzi*.

Waleckx E, Suarez J, Richards B, Dorn PL. 2014. *Triatoma sanguisuga* blood meals and potential for Chagas disease, Louisiana, USA. *Emerg Infect Dis*. **20:**2141–2143.

This study provides evidence that *Triatoma sanguisuga* represents a threat to both humans and animals in Louisiana, where contact with *Trypanosoma cruzi* has been documented in 55% of bugs checked. Of note is the fact that almost 40% of these bugs had fed on human blood. Although this study provides evidence of frequent vector-human-*T. cruzi* contact in Louisiana, these findings are not consistent throughout the United States. Raccoons and dogs, as well as woodrats, serve as reservoir animals.

Sanchez DR, Traina MI, Hernandez S, Smer AM, Khamag H, Meymandi SK. 2014. Chagas disease awareness among Latin American immigrants living in Los Angeles, California. *Am J Trop Med Hyg* **91:**915–919.

The number of cases of Chagas' disease in the United States has been estimated to be from 150,000 to over 300,000. **While the majority of these cases originated in Latin America, there is a wide range of understanding of this disease among immigrants currently living in Los Angeles.** In a study of 2,677 immigrants who had lived in Latin America for a minimum of 6 months, 62% had actually seen triatomid bugs in Latin America, and 27% reported being bitten at least once a year. However, 82% were totally unaware of Chagas' disease. Those who had at least heard of the disease reported it was not serious (81%). However, on a more positive note, those who were aware of the disease indicated they preferred to be tested and treated. Unfortunately, the data indicated that in this particular group of immigrants, the overall awareness of Chagas' disease was very limited. Another important issue to consider is that this infection is now endemic in many areas of the southern United States; therefore, we expect to see future cases in the population in individuals who have never been outside the United States.

Intestinal Nematodes

Hookworm

Croese J, Giacomin P, Navarro S, Clouston A, McCann L, Dougall A, Ferreira I, Susianto A, O'Rourke P, Howlett M, McCarthy J, Engwerda C, Jones D, Loukas A. 2014. Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. *J Allergy Clin Immunol* **135:**508–516.

Celiac disease is a common gluten-sensitive autoimmune disorder of the small intestine that occurs in people who are genetically predisposed. Absorption of nutrients can be severely compromised, villous atrophy is seen, and a gluten-free diet is recommended. However, compliance with this regimen can be very difficult to achieve. Since parasitic helminths have been used for the treatment of inflammatory disorders, the authors assessed the impact of experimental *Necator americanus* infection on celiac disease patients who received increased gluten over time. **The study revealed no decrease in the villous blunting; however, the overall quality of life improved, T cells (IFNy) were reduced, and regulatory T cells increased. Overall, this approach maintained or improved gluten toxicity indicators in this patient group.**

Strongyloides stercoralis

Puthiyakunnon S, Boddu S, Li Y, Zhou X, Wang C, Li J, Chen X. 2014. Strongyloidiasis: an insight into its global prevalence and management. *PLoS Negl Trop Dis* **8:**e3018. doi:10.1371/journal.pntd.0003018.

Strongyloides stercoralis infects more than 100 million people worldwide. *Strongyloides* have a free-living stage within the life cycle, as well as an autoinfective cycle. Patient presentations vary from those who are asymptomatic to those with the fatal hyperinfection or disseminated infection syndromes. **Important risk factors include immunosuppression related to corticosteroid use, infection with human T-lymphotropic virus or human immunodeficiency virus, hematology malignancies, and allograft transplant recipients.** Strongyloidiasis is classified as one of the neglected tropical diseases. Diagnostic procedures tend to miss the infection (low parasitemia), and the infection may not be suspected (nonspecific clinical symptoms). Improved molecular methods and a more comprehensive understanding of this infection are critical for good clinical outcomes.

Salvador F, Sulleiro E, Sánchez-Montalvá A, Saugar JM, Rodríguez E, Pahissa A, Molina I. 2014. Usefulness of *Strongyloides stercoralis* serology in the management of patients with eosinophilia. *Am J Trop Med Hyg* **90:**830–834.

It is important to remember that *Strongyloides stercoralis* infections continue to be seen in areas of no endemicity. Use of the *S. stercoralis* serology in patients presenting with eosinophilia has become more widely recommended. In this study 147 patients were involved; 28 had HIV, and 89 (60.5%) patients had a positive *S. stercoralis* serology. However, using diagnostic tests that are somewhat insensitive, larvae were detected only in 15 (10.2%) patients. Eighty patients were given ivermectin 200 μg/kg/day for 2 days; 32 patients were seen for follow-up after 6 months. Twenty-six patients (81.3%) had a negative serology 6 months after treatment. Also, the EIA posttreatment to pretreatment decreased to 0.6, and 11 (34.4%). Based on this and other published data, ***S. stercoralis* serology is valuable in supporting the presumptive diagnosis and for subsequent testing post-therapy.**

Anatrichosoma spp.

Eberhard ML, Hellstein JW, Lanzel EA. 2014. Zoonotic anatrichosomiasis in a mother and daughter. *J Clin Microbiol* **52:**3127–3129.

Human infections with *Anatrichosomia* spp. (small tichuroid nematodes) are rare and are usually associated with oral lesions. The complete life cycle remains unknown, although it may include an arthropod intermediate host. This case reports infection in a mother and daughter. Both patients were seen after a 10-week history of multiple painful oral ulcers. Biopsy tissue examination showed small, coiled trichuroid nematodes with morphological features compatible with *Anatrichosoma* spp. Morphology revealed stichocytes, which were visible in the anterior end, a small tubular esophagus, and the presence of two bacillary bands. In a previous case reported by these authors, the patient was treated with 100 mg mebendazole twice daily for 20 days; this therapy was successful.

Tissue Nematodes

Angiostrongylus cantonensis

York EM, Butler CJ, Lord WD. 2014. Global decline in suitable habitat for *Angiostrongylus* (= *Parastrongylus*) *cantonensis*: the role of climate change. *PLoS One* **9:**e103831. doi:10.1371/journal.pone.0103831.

The parasite *Angiostrongylus* (= *Parastrongylus*) *cantonensis* is an invasive helminth that causes eosinophilic meningitis in humans. *A. cantonensis* has been reported from more than 30 countries worldwide. Given the health risks and serious disease sequelae, it is important to identify habitats where climate currently supports *A. cantonensis* or those in which these parasites could become established in the future. Although some evidence from this study indicates an overall decline in suitable habitats, these results do not agree with other published studies. Overall evidence suggests that the prevalence of parasitic pathogens will continue to increase throughout this century. **Based on this evidence, *A. cantonensis* will most likely continue to expand its geographic range; however, overall climate change may reduce the total geographic area of suitable climatic conditions throughout the 21st century.**

Stockdale-Walden HD, Slapcinsky J, Qvarnstrom Y, McIntosh A, Bishop HS, Rosseland B. 2015. *Angiostrongylus cantonensis* in introduced gastropods in southern Florida. *J Parasitol* **101:**156–159.

This infection has been reported throughout the world in many countries and is prevalent in Asia, the Pacific Islands, and the Caribbean. Human infection begins with the accidental ingestion of infective larvae in several species of slugs, snails, or land planarians. Ingestion of infected raw paratenic hosts, including fish, amphibians, reptiles, crustaceans, and vegetables contaminated with larvae, also leads to infection of the human host. In the natural life cycle, infective larvae have also been found in land crabs, coconut crabs, and freshwater prawns, which are often consumed raw in the Pacific Islands. In summary, human infection can originate through the following: ingestion of L3 larvae in raw or undercooked intermediate or paratenic hosts, drinking infected water, oral contact with hands contaminated with mollusk larvae, or possibly through the skin. There are three major forms of human angiostrongyliasis: meningitic, encephalitic, and ocular; the majority of patients exhibit the meningitic form. Although rare, the infection can be fatal. Symptoms include severe headache, convulsions, weakness of the limbs, paresthesia, vomiting, constipation, nausea, anorexia, facial paralysis, neck stiffness, and fever. **The presence of definitive and intermediate hosts strongly suggests the presence of *A. cantonensis* in south Florida.**

Anisakiasis

Baird FJ, Gasser RB, Jabbar A, Lopata AL. 2014. Foodborne anisakiasis and allergy. *Mol Cell Probes* **28:**167–174.

Anisakiasis in humans is a disease caused by *Anisakis* spp. (*Nematoda*) and often presents as acute gastroenteritis, which is the most commonly recognized clinical syndrome and which mimics bacterial or viral gastroenteritis. Since the presence of anisakid larvae in infected marine fish is worldwide, the risk of anisakiasis in developed countries is probably under-recognized and underreported. **This disease not only causes serious symptoms in the gastrointestinal tract, but it is well documented that these infections can also cause serious allergic reactions in patients who have become sensitized.** Allergic symptoms range from urticaria and isolated angioedema to anaphylaxis. It has been highly recommended that serologic testing for antibody to *Anisakis* spp. should be performed on any patient where the presumptive diagnosis is eosinophilic gastroenteritis.

Baylisascaris procyonis

Page K, Smyser TJ, Dunkerton E, Gavard E, Larkin B, Gehrt S. 2014. Reduction of *Baylisascaris procyonis* eggs in raccoon latrines, suburban Chicago, Illinois, USA. *Emerg Infect Dis* **20:**2137–2140.

Baylisascaris procyonis is the raccoon ascarid, has a normal ascarid-like life cycle, causes a very serious zoonotic disease in humans (larval migrans), and is most often reported in North America. Adult worms produce 150,000 eggs/worm/day, and infected raccoons can shed as many as 250,000 eggs per gram of feces. This level of egg production can lead to significant environmental contamination of the soil, particularly in areas where the raccoons defecate (raccoon latrines). Although many control measures have been tried, most have proven to be unsuccessful. **However, this study reports on the success of an antihelmintic baiting approach for the reduction of environmental contamination with *B. procyonis* eggs.**

Capillaria hepatica (*Calodium hepaticum*)

Fuehrer HP. 2014. An overview of the host spectrum and distribution of *Calodium hepaticum* (syn. *Capillaria hepatica*). Part 2. *Mammalia* (excluding *Muroidea*). *Parasitol Res* **113:**641–651.

Calodium hepaticum (syn. *Capillaria hepatica*) is a zoonotic nematode found worldwide with low host specificity; it is found in

25 families of nonrodent mammals and is primarily found in the liver. Various animal groups involved are the *Insectivora*, *Chiroptera*, *Lagomorpha*, *Artiodactyla*, *Perissodactyla*, *Hyracoidea*, *Marsupialia*, *Carnivora*, and *Primates*, including humans. There is a direct life cycle, which includes egg ingestion, hatching within the cecum, and liver invasion via the portal veins. Adult worms remain in the liver for up to 60 days. Egg ingestion of infected animal livers results in spurious infections, and eggs are passed in the feces. **Diagnosis is primarily based on liver biopsy and autopsies; because the eggs of this helminth can be easily confused with those of other genera/species, infection reports may be somewhat inaccurate.**

Gnathostoma spp.

Cole RA, Choudhury A, Nico LG, Griffin KM. 2014. *Gnathostoma spinigerum* in live Asian swamp eels (*Monopterus* spp.) from food markets and wild populations, United States. *Emerg Infect Dis* **20:**634–642.

In various areas within Asia, wild and aquaculture-grown swamp eels (*Synbranchidae*: *Monopterus* spp.) are a common food source for humans, a practice which leads to gnathastomiasis. Live Asian swamp eels are imported to U.S. markets, and populations in the wild are documented in several states. The authors examined 47 eels from markets and 67 wild-caught specimens for the presence of *Gnathostoma*. Thirteen (27.7%) *Monopterus cuchia* eels from markets were infected with 36 live *Gnathostoma spinigerum* AL3: most (21) were in the liver (58.3%), 7 (19.4%) in muscle, 5 (13.8%) in the gastrointestinal tract, and 3 (8.3%) in kidneys. Species from North America included three (4.5%) wild-caught *Monopterus albus* eels that were infected with five *Gnathostoma turgidum* AL3 in muscle, and one *Gnathostoma lamothei* AL3 that was found in a kidney. In U.S. ethnic food markets, imported swamp eels from Asia and native and nonnative fishes are commonly maintained alive for purchase. Often, customers will ask to have the fish processed before they leave the market. It is also known that some of these fish are released into the wild, thus potentially introducing nonendemic parasites into native habitats.

Toxocara spp.

Lee RM, Moore LB, Bottazzi ME, Hotez PJ. 2014. Toxocariasis in North America: a systematic review. *PLoS Negl Trop Dis* **8:**e3116. doi:10.1371/journal.pntd.0003116.

Toxocariasis is an important neglected tropical disease caused by *Toxocara canis* and *Toxocara cati* that can be seen in humans as visceral or ocular larva migrans or as asymptomatic disease. This infection is of public health concern, and illness can cause serious sequelae. The authors conducted a systematic PubMed review of the literature to determine the disease prevalence in North America. They reviewed 18 articles with original prevalence, incidence, or case data for toxocariasis. Prevalence estimates ranged from a low of 0.6% in a Canadian Inuit community to a high of 30.8% in Mexican children presenting with asthma. Risk factors have been identified as African-American race, poverty, male sex, age under 18, living in the southern United States, and dog or cat ownership or children playing in uncovered sandboxes where animals tend to defecate. This disease is definitely considered in "no pet beaches" where sand, sun, and moisture provide an excellent environment for these nematodes. Additional studies from Mexico have identified other high-risk groups including waste pickers, asthmatic children, and inpatient psychiatry patients. Although a thorough understanding of these infections remains incomplete, they produce very serious sequelae, particularly in young children.

Trichinella spp.

Hill DE, Dubey JP, Baroch JA, Swafford SR, Fournet VF, Hawkins-Cooper D, Pyburn DG, Schmit BS, Gamble HR, Pedersen K, Ferreira LR, Verma SK, Ying Y, Kwok OC, Feidas H, Theodoropoulos G. 2014. Surveillance of feral swine for *Trichinella* spp. and *Toxoplasma gondii* in the USA and host-related factors associated with infection. *Vet Parasitol* **205:**653–665.

Trichinella spp. and *Toxoplasma gondii* are found worldwide and are significant zoonotic parasites that tend to infect warm-blooded animals and humans. While pigs are the main host for *Trichinella spiralis*, many animals such as pigs, chickens, sheep, and goats can be infected with *T. gondii*. It has been documented that wildlife infections have a higher incidence than those seen in domestic animals. Feral swine are reservoirs in the sylvatic habitat for *Trichinella* spp. and *T. gondii* and are infection sources for carnivores that are found within the same areas as domestic pigs. Feral swine in direct contact with domestic pigs can lead to infection through cannibalism.

Between 2006 and 2010, the authors conducted a serological survey in the United States to obtain estimates regarding *Trichinella* spp. and *T. gondii* and potential infection risk factors for feral swine. Serum samples (3,247) were tested, and results are available from 26 states. Seroprevalence data were 3.0% for *Trichinella* spp. and 17.7% for *T. gondii*. Distribution areas for both parasites were similar and tended to concentrate in the South and the Midwest. A subsequent survey of 984 feral pigs between 2012 and 2013 revealed a 2.9% rate for *Trichinella* spp. and 28.4% for *T. gondii*. Tongues (330) were sampled from the 984 animals, and 1.81% of the feral pigs were confirmed positive for *T. spiralis*. **With the potential for overlapping feral pig and domestic pig habitat/production facilities, it is quite possible that *Trichinella* spp. and *T. gondii* could be introduced into domestic pigs in the United States.**

Filarial Nematodes

Paniz-Mondolfi AE, Gárate T, Stavropoulos C, Fan W, González LM, Eberhard M, Kimmelstiel F, Sordillo EM. 2014. Zoonotic filariasis caused by novel *Brugia* sp. nematode, United States, 2011 [letter]. *Emerg Infect Dis* **20:**1248–1250.

Brugia spp. filarial worms that normally infect nonhuman vertebrates have been identified as rarely causing human zoonotic infection. **While lymphatic filariasis caused by *Brugia malayi* and *Brugia timori* occurs in Asia, most zoonotic infections with other**

animal species of *Brugia* are documented from the northeastern United States or South America. One case report occurred in a New York City resident with no relevant travel history. This patient suffered from right groin swelling and had a 12% eosinophilia, and the biopsy revealed intralymphatic nematodes with living microfilariae. Subsequently, the patient complained of inguinal swelling; on repeat biopsy no additional worms were detected. However, the patient was given oral doxycycline for 6 weeks, followed by ivermectin and albendazole, with no further problems for over 2 years. Since many reported patient infections are asymptomatic or confirmed as incidental findings, the overall disease prevalence remains undetermined. Also, in areas of disease endemicity, cases are unlikely to be categorized as zoonotic or conventional. The majority of cases were reported from the Northeast, including New York (eight), Massachusetts, Pennsylvania, Connecticut, and Rhode Island (three); single cases have been identified in Michigan, Ohio, North Carolina, Oklahoma, New Jersey, Louisiana, Florida, and California. Outside of the United States, three cases have been reported from South America (Colombia, Brazil, Peru) and one from Africa (Ethiopia). *Brugia leporis* is seen in rabbits in the northeastern United States, *Brugia beaveri* is found in raccoons and bobcats in the southern United States, and *Brugia guyanensis* is found in the coatimundi and other vertebrates in South America.

Hopkins DR, Ruiz-Tibin E, Eberhard ML, Roy SL. 2014. Progress toward global eradication of dracunculiasis: January 2013-June 2014. *MMWR Morb Mortal Wkly Rep* **63:**1050–1054.

The number of new cases of dracunculiasis (Guinea worm disease) worldwide each year has decreased from an estimated 3.5 million in 1986 (the year global elimination as a goal was announced) to 148 in 2013. The number of cases worldwide in 2013 (148) declined by 73% compared with 2012 (542), and by 71% during January–June 2014 (27) compared with January–June 2013 (92). Transmission remains endemic in four countries (Mali, Chad, Ethiopia, Sudan), with South Sudan accounting for 70% of all reported cases during January–June 2014. Although the original eradication dates were missed, it is anticipated that eradication is likely during the next year or two.

Mombo-Ngoma G, Mackanga JR, Basra A, Capan M, Manego RZ, Adegnika AA, Lötsch F, Yazdanbakhsh M, Gonzalez R, Menendez C, Mabika B, Matsiegui PB, Kremsner PG, Ramharter M. 2015. *Loa loa* infection in pregnant women, Gabon. *Emerg Infect Dis* **21:**899–901.

A prospective study of pregnant women in Central Africa revealed a high prevalence of *Loa loa* infection. Although little information is available on the epidemiology of this infection in pregnancy, **the invasion of microfilariae into the intervillous space of the placenta was confirmed in this study**. Additional clinical studies are under way to clarify these findings.

Hassan KH, Bolcen S, Kubofcik J, Nutman TB, Eberhard ML, Middleton K, Wekesa JW, Ruedas G, Nelson KJ, Dubielzig R, De Lombaert M, Silverman B, Schorling JJ, Adler PH, Unnasch TR, Beeler ES. 2015. Isolation of *Onchocerca lupi* in dogs and black flies, California, USA. *Emerg Infect Dis* **21:**789–796.

Data from this study confirm that canine infections with *Onchocerca lupi* are ongoing in California; thus, cats and humans could also be potentially infected. It has been suggested that other canids such as coyotes could serve as reservoir animals. **Although it is unknown whether common heartworm medications used for dogs are effective against the larvae of *O. lupi*, their use may help prevent infection in pets.**

Otranto D, Giannelli A, Latrofa MS, Dantas-Torres F, Trumble NS, Chavkin M, Kennard G, Eberhard ML, Bowman DD. 2015. Canine infections with *Onchocerca lupi* nematodes, United States, 2011-2014. *Emerg Infect Dis* **21:**868–871.

Since canine onchocerciasis caused by *Onchocerca lupi* has been documented in Arizona, California, Colorado, Florida, Minnesota, New Mexico, and Utah, it has been recommended that further studies be expanded throughout the United States. **The potential for dogs to serve as reservoir hosts for possible human infections has taken on more importance as additional canine and human cases are reported.**

Intestinal Cestodes

Taenia saginata

Tembo A, Craig PS. 2014. *Taenia saginata* taeniosis: copro-antigen time-course in a voluntary self-infection. *J Helminthol* **19:**1–8.

Although infection with *Taenia saginata* is worldwide where beef is consumed, relatively little is known about the early stage in humans. While copro-antigen detection is helpful in patient screening for *T. solium*, there are essentially no data related to early *T. saginata* infection. A self-infection with *T. saginata* was initiated and monitored for 6 months using a copro-antigen enzyme-linked immunosorbent assay (ELISA) developed by the authors. The first proglottid was seen on day 86 postinfection and was followed by almost daily release of proglottids (range 1–8) until praziquantel therapy on day 180 postinfection. For the first 10 weeks the patient remained asymptomatic. By weeks 15–19 postinfection, abdominal discomfort was the main clinical finding, while involuntary proglottid discharge continued during the infection period. Copro-antigens were detected 2 weeks before proglottids were detected, remained highly elevated until treatment, and reverted to negative 4 days posttherapy. **Although copro-antigen ELISA is an appropriate and sensitive diagnostic test in *T. saginata* infections with gravid worms, it is much less sensitive prior to the release of proglottids.**

Taenia solium

**O'Neal SE, Moyano LM, Ayvar V, Rodriguez S, Gavidia C, Wilkins PP, Gilman RH, Garcia HH, Gonzalez AE, Cysticercosis

Working Group in Peru. 2014. Ring-screening to control endemic transmission of *Taenia solium*. *PLoS Negl Trop Dis*. **8**:e3125. doi:10.1371/journal.pntd.0003125.

In many areas of the world, including Latin America, Asia, and Africa, *Taenia solium* is the third leading cause of preventable epilepsy. Elimination of infection with the adult tapeworms would help reduce exposure to the infective eggs (cause of human/pig cysticercosis). The authors implemented a detection/therapy program among household personnel (1,058 in the study site, 753 in the control site) located in a defined radius around heavily infected pigs (ring screening) (1,444 from the study site, 966 from the control site). After 1 year, including therapy for confirmed human cases with adult *T. solium*, the authors documented a 41% reduction in seroprevalence in the study site, while the control site remained the same. **Although this screening approach was very effective, empiric therapy using niclosamide for humans and oxfendazole for pigs remains another option based on drug safety records.**

Garcia HH, Gonzales I, Lescano AG, Bustos JA, Pretell EJ, Saavedra H, Nash TE, Cysticercosis Working Group in Peru. 2014. Enhanced steroid dosing reduces seizures during antiparasitic treatment for cysticercosis and early after. *Epilepsia* **55**:1452–1459.

Neurocysticercosis is one of the leading causes of seizures and epilepsy in areas worldwide where *Taenia solium* is endemic. In some countries the prevalence exceeds 10%, and neurocysticercosis is responsible for up to 50% of late-onset epilepsy cases. Patient responses vary from being asymptomatic with overall parasite tolerance to a severe inflammatory response. Antiparasitic therapy and subsequent death of the organisms may lead to serious inflammatory reactions by the patient. In many cases, steroids are given at the same time to avoid these inflammatory complications. The authors made the assumption that increased steroid dosing would decrease treatment-associated seizures in neurocysticercosis patients. **Data confirmed that dexamethasone resulted in fewer seizures for the first 21 days during and early after antiparasitic treatment with albendazole; however, this finding was not consistent during the first 11–42 days, which was the preset primary selected time frame for the study outcome assessment of seizure days and patients with seizures.**

Cantey PT, Coyle CM, Sorvillo FJ, Wilkins PP, Starr MC, Nash TE. 2014. Neglected parasitic infections in the United States: cysticercosis. *Am J Trop Med Hyg* **90**:805–809.

The larval forms of *Taenia solium* are called cysticerci; they have been recovered from all areas of the human body, produce symptoms that depend on the particular body site involved, and can cause fatal disease. The presence of cysticerci in the brain represents the most frequent parasitic infection of the human nervous system and the most common cause of adult-onset epilepsy throughout the world. Patients with neurocysticercosis may have seizures or increased intracranial pressure. Although many emigrants in the United States came from cysticercosis-endemic areas such as Mexico and Latin America and autochthonous transmission has been documented in the United States, information regarding the disease epidemiology in this country is limited. A number of things are recommended, including mandatory reporting, the determination of better prevalence/hospitalization data, development of prospective data on mortality risks, improved diagnostic testing, and clarification of treatment and patient management recommendations.

O'Keefe KA, Eberhard ML, Shafir SC, Wilkins P, Ash LR, Sorvillo FJ. 2014. Cysticercosis-related hospitalizations in the United States, 1998-2011. *Am J Trop Med Hyg* **92**:354–359.

The authors reviewed inpatient records for cysticercosis using the Nationwide Inpatient Sample for 1998–2011. There were 33,060 cysticercosis-related hospitalizations during this time frame (8.03/million). Key statistics included male (54.8%), Hispanic (62.0%), age 18–44 (58.8%), occurred in the western U.S. (45.1%), and deaths (459; fatality rate of 1.4%). The authors recommend that cysticercosis should be a nationally reportable disease, and the overall numbers are greater than previously thought.

Del Brutto OH, Salgado P, Lama J, Del Brutto VJ, Campos X, Zambrano M, Garcia HH. 2015. Calcified neurocysticercosis associates with hippocampal atrophy: a population-based study. *Am J Trop Med Hyg* **92**:64–68.

Although calcified cysticerci in the brain parenchyma in many cases have been considered clinically insignificant, in areas of endemicity there may be large numbers of patients with **epilepsy and neurocysticercosis**. There has also been a link between calcified cysticerci and **hippocampal atrophy**. In a recent study of patients (≥60) in Ecuador, there was a definite association between calcified cysticerci and hippocampal atrophy. Subsequent studies are being planned for younger individuals to determine (i) if calcified cysticerci are related to the development of hippocampal atrophy over time and (ii) whether hippocampal atrophy is linked to temporal lobe epilepsy.

Tissue Cestodes

Echinococcus spp.

Rogan MT, Bodell AJ, Craig PS. 2014. Post-encystment/established immunity in cystic echinococcosis: is it really that simple? *Parasite Immunol* **37**:1–9.

Although multiple studies have investigated immunity to larval cestodes, clarification of a two-phase system is probably too simplistic. *Echinococcus* spp. exhibit different developmental changes depending on the host and include growth, regeneration, and deterioration. Cyst classification based on clinical grounds is undergoing change. **Based on medical and veterinary information, the authors have proposed that the "established" phase for *Echinococcus granulosus* could be further divided into the following phases: maturing, stable, unstable, and degenerative.**

Martel G, Ismail S, Begin A, Vandenbroucke-Menu F, Lapointe R. 2014. Surgical management of symptomatic hydatid liver disease: experience from a Western centre. *Can J Surg* **57:**320–326.

The majority of hydatid cysts occur in the liver, primarily in the right lobe. Symptoms include chronic abdominal discomfort, occasionally with a palpable or visible abdominal mass, pain, hepatomegaly, cholestasis, biliary cirrhosis, portal hypertension, and ascites. Treatment options for hydatid disease include medical treatment with albendazole or mebendazole for smaller cysts, conservative surgery (drainage, some tissue removal, cavity management), and radical surgery (liver resection). Based on World Health Organization recommendations, "surgery was [the] recommendation for large liver cysts; symptomatic cysts; cysts at risk of rupture; infected cysts; and cysts demonstrating or at risk for developing fistulas into the biliary tree, diaphragm/pleural cavity or other vital organs." The authors reported that 67% of patients received drugs prior to surgery, 87% after surgery, and 60% pre- and postsurgery. Eighty-three percent underwent liver resection, while 18% underwent conservative therapy. **Study data reinforced the fact that liver surgery for echinococcosis in North America is somewhat rare; however, the data also documented a large number of complicated cysts in terms of clinical management.** This publication provides an excellent discussion of the pros and cons of hydatid cyst management in North America.

Piccoli L, Tamarozzi F, Cattaneo F, Mariconti M, Filice C, Bruno A, Brunetti E. 2014. Long-term sonographic and serological follow-up of inactive echinococcal cysts of the liver: hints for a "watch-and-wait" approach. *PLoS Negl Trop Dis* **8:**e3057. doi:10.1371/journal.pntd.0003057.

Cystic echinococcosis is one of the neglected parasitic diseases and can have a wide range of presentations in terms of involved organs, varying cyst stages, and patient clinical symptoms, from those who are asymptomatic to those with severe sequelae. Although radical surgery, minor surgery, and chemotherapy (with or without surgical intervention) are the three approaches to treatment, extensive evaluation of these approaches has been lacking. **With the advent of ultrasound options, those with experience in treating echinococcosis have recommended that uncomplicated inactive cysts should be monitored but not treated.** The authors report that over a 19-year period, 97.4% of their patients with inactive cysts were monitored for 2 years with no evidence of reactivation. Their data were also confirmatory in suggesting that serologic testing was helpful but could not be used alone to guide the clinical management of these patients.

Grenouillet F, Umhang G, Arbez-Gindre F, Mantion G, Delabrousse E, Millon L, Boué F. 2014. *Echinococcus ortleppi* infections in humans and cattle, France. *Emerg Infect Dis* **20:**2100–2102.

Echinococcus orleppi has a dog-cattle cycle with limited pathogenicity for human infections (eight reported cases previously). Although most reports originate from South America, limited reports have come from other locations. Recently, human and cattle infections have been reported from France. **Because most diagnostic reagents are prepared from *Echinococcus granulosus*, negative results in patients with *Echinococcus orleppi* may result in underreporting of this infection.**

Stijnis K, Dijkmans AC, Bart A, Brosens LAAS, Muntau B, Schoen C, Barth TF, van Gulik T, van Gool T, Grobusch MP, Tappe D. 2015. *Echinococcus vogeli* in immigrant from Suriname to the Netherlands. *Emerg Infect Dis* **21:**528–530.

It is known that echinococcosis infections (polycystic *Echinococcus vogeli*, unicystic *Echinococcus oligarthrus*) have been confirmed as emerging infections in South America, primarily Brazil. The life cycle of *E. vogeli* includes the intermediate host (pacas) and the final host (bush dogs). A recent case has been identified in a patient seen in the Netherlands. The patient was a 48-year-old female who presented for retrosternal pain; she had immigrated to the Netherlands from Suriname approximately 20 years before. Although most physical and laboratory findings were reported as normal, abdominal ultrasonography demonstrated a mixed solid/liquid lesion that suggested a biliary cystadenoma or an echinococcal cyst. The patient's serology was strongly positive; subsequently, surgical resection was performed, resulting in removal of an 8-cm polycystic tumor. While the original diagnosis was suggestive of *Echinococcus multilocularis* or *Echinococcus granulosus*, further examination using PCR revealed 99 to 100% homology with *E. vogeli*. **This and other cases indicate that there may be more undiagnosed cases of *E. vogeli* in immigrants from South America. Based on a presumptive diagnosis, these patients would receive preoperative and long-term therapy with albendazole.** Excellent images of rostellar hooks from the protoscolices can be found in the same issue of the journal on page 458 ("*Polycystis echinococcosis* in pacas, Amazon region, Peru").

Spirometra spp.

Cheng KB, Gao BL, Liu JM, Xu JF. 2014. Pulmonary sparganosis mansoni: a case report from a nonendemic region. *J Thorac Dis* **6:**E120–E124.

The larval forms (spargana) of *Spirometra mansonoides* can be found in the subcutaneous connective tissue and between the muscles in frogs, lizards, snakes, birds, and certain mammals. Sparganosis is most common in China, Japan, and Southeast Asia. This case involved a 32-year-old female who had no relevant travel history and who was misdiagnosed as having eosinophilic pneumonia. Although she was treated for approximately 2 months with corticosteroids, she remained symptomatic. Her computed tomography scan demonstrated lesions, and she was admitted for additional workups. Per her medical history, she commonly ate raw seafood, as well as various types of raw frogs. A serologic result revealed antibodies consistent with the diagnosis of pulmonary sparganosis, and she was successfully treated with praziquantel. **Although this infection is rare, a thorough medical history is required to diagnose sparganosis in either endemic or nonendemic areas where the patient has a history of ingesting raw frogs or snakes.**

Lung Trematodes

Paragonimus spp.

Xia Y, Ju Y, Chen J, You C. 2014. Hemorrhagic stroke and cerebral paragonimiasis. *Stroke* **45:**3420–3422.

Hemorrhagic cerebral paragonimiasis patients (10) were reviewed for clinical findings, diagnosis, and therapy. Patients underwent various diagnostic procedures including brain computed tomography scans (10), magnetic resonance imaging (9), computed tomography angiography (4), magnetic resonance angiography (4), digital subtraction angiography (4), cerebrospinal fluid cytology examination (7), and follow-up (9) for 12 to 62 months. In reviewing 27 cerebral paragonimiasis cases overall, 10/27 (37%) had hemorrhagic complications. As would be expected, symptoms included severe headaches, blurred vision, tinnitus, vomiting, hemiparalysis, and epilepsy. While four cases underwent surgery and symptoms improved, central nervous system deficits remained in three of these patients. **With cerebral paragonimiasis, it is imperative that treatment be initiated as quickly as possible to decrease the risk of stroke complications.**

Song J, Hong G, Song JU, Kim W, Han SG, Ko Y, Chang B, Jeong BH, Eom JS, Lee JH, Jhun BW, Jeon K, Kim HK, Koh WJ. 2014. A case of pleural paragonimiasis confused with tuberculous pleurisy. *Tuberc Respir Dis (Seoul)* **76:**175–178.

Paragonimus westermani causes pleuropulmonary disease in humans. Symptoms can often mimic other disorders such as tuberculosis or cancer. The patient was a 38-year-old man with pleural effusion (radiography) with pleural thickening (computed tomography scan). Prior to the computed tomography scans, the patient was seen after the development of a right pneumothorax; based on a chest radiograph and other clinical findings, he was started on empirical therapy for tuberculosis (isoniazid, rifampin, ethambutol, pyrazinamide). After continued failure to improve, surgery was performed, and pathology findings included granulomatous inflammation with helminth eggs. Prior therapy was withdrawn, and the patient was started on praziquantel. After posttherapy and follow-up (2 years), the patient remains symptom free. **In areas where tuberculosis and paragonimiasis are endemic, it is important to consider pleural paragonimiasis as a potential diagnosis in patients with pleural effusion.**

Blood Trematodes (Schistosomes)

Scott PL, Lim RM, Dukes JP, Cook RT, Walker AJ, Kirk RS. 2014. Identification of a major causative agent of human cercarial dermatitis, *Trichobilharzia franki* (Müller and Kimmig 1994), in southern England and its evolutionary relationships with other European populations. *Parasit Vectors* **7:**277.

Many species of bird schistosomes have been documented to cause human cercarial dermatitis (CD). These schistosomes are seen worldwide, and CD is thought to be an emerging infectious disease, either through the spread of areas of endemicity and/or in more accurate recognition of the disease. CD follows skin penetration by cercariae released from freshwater snail intermediate hosts, and the allergic reaction may be due partly to previous host exposure. Few symptoms are associated with primary exposure, but both humoral and cellular immune responses are elicited on subsequent exposure. After cercarial skin penetration, petechial hemorrhages with edema and pruritus occur. The subsequent maculopapular rash, which may become vesicular, may last 36 h or more. Dermatitis is a constant feature of human infection with avian schistosomes, with cercarial death occurring in the subcutaneous tissues and immediate hypersensitivity reactions occurring at the invasion sites. *Trichobilharzia* spp. are the most common cause of CD and have been confirmed in outbreaks in the United States and Europe. Based on molecular testing, *Trichobilharzia franki* was identified in the United Kingdom, and these isolates were closely related to populations from France. CD must be included in the list of differential diagnosis of skin lesions, especially in areas of endemicity where migratory waterfowl influence potential habitats.

El Scheich T, Holtfreter MC, Ekamp H, Singh DD, Mota R, Hatz C, Richter J. 2014. The WHO ultrasonography protocol for assessing hepatic morbidity due to *Schistosoma mansoni*. Acceptance and evolution over 12 years. *Parasitol Res* **113:**3915–3925.

This study reviews the worldwide acceptance of the World Health Organization (WHO) ultrasound protocol for the evaluation of hepatosplenic disease due to *Schistosoma mansoni* published in 2000. Sixty studies involving 37,424 patients from 15 countries were included in the study. Use of the WHO procedure increased from 43.75% during 2001 to 2004 to 84.61% in 2009 to 2012. Results using the pictorial image approach are reported in 38 of 41 reviewed publications, measurements of portal branch walls were reported in 19 of 41, and results are reported in 2 of 41. The practical usefulness of the pictorial approach of the WHO protocol is confirmed by its wide acceptance. **The WHO pictorial approach has gained wide acceptance based on reproducibility, evaluation of pathology, and comparisons between different locations.**

Doumbo S, Tran TM, Sangala J, Li S, Doumtabe D, Kone Y, Traoré A, Bathily A, Sogoba N, Coulibaly ME, Huang CY, Ongoiba A, Kayentao K, Diallo M, Dramane Z, Nutman TB, Crompton PD, Doumbo O, Traore B. 2014. Coinfection of long-term carriers of *Plasmodium falciparum* with *Schistosoma haematobium* enhances protection from febrile malaria: a prospective cohort study in Mali. *PLoS Negl Trop Dis* **8:**e3154. doi:10.1371/journal.pntd.0003154.

The authors evaluated the impact of baseline *Schistosoma haematobium* infection, baseline *Plasmodium falciparum* infection, and coinfection with both organisms on the possible risk of febrile malaria. Patients who were found to be positive for *S. haematobium* were treated within a few weeks with praziquantel. Malaria episodes were tracked for 7 months. After adjustment for a number of factors, baseline *P. falciparum* mono-infection ($n = 254$) and coinfection ($n = 39$) were highly related to protection from febrile malaria. The protective effect of coinfection was greatest in patients with no anemia. Coinfection was also linked with lower parasitemia during the first febrile episode. **This study confirmed that coinfection with *S. haematobium* and *P. falciparum* substantially decreases the risk of febrile malaria in asymptomatic carriers of *P. falciparum*.**

Ross AG, Olveda RM, Chy D, Olveda DU, Li Y, Harn DA, Gray DJ, McManus DP, Tallo V, Chau TN, Williams GM. 2015. Can mass drug administration lead to the sustainable control of schistosomiasis? *J Infect Dis* **211:**283–289.

In the Philippines, the current control approach for schistosomiasis is yearly mass drug administration (MDA) with 40 mg/kg of praziquantel in all areas with a prevalence of ≥10%. Despite schistosomiasis control efforts for over 30 years, including MDA, the mean prevalence of schistosomiasis among 10,435 subjects was 27.1%. High morbidity findings included left-lobe liver enlargement (89.3%), grade II/III liver parenchyma fibrosis (25%), and splenomegaly (13.3%). **MDA alone was unable to control the prevalence, intensity of infection, or morbidity of schistosomiasis.**

Santos J, Gouveia MJ, Vale N, Delgado Mde L, Gonçalves A, da Silva JM, Oliveira C, Xavier P, Gomes P, Santos LL, Lopes C, Barros A, Rinaldi G, Brindley PJ, da Costa JM, Sousa M, Botelho MC. 2014. Urinary estrogen metabolites and self-reported infertility in women infected with *Schistosoma haematobium*. *PLoS One* **9:**e96774. doi:10.1371/journal.pone.0096774.

It is suspected that urogenital schistosomiasis caused by *Schistosoma haematobium* may be the most neglected tropical disease in sub-Saharan Africa, with over 120 million cases caused by this infection. Based on prior studies involving metabolic pathways in cancer caused by estrogens, the authors developed a study of female residents in Angola, where urogenital schistosomiasis is endemic. Women and girls (93) provided data on urinary, urogenital, and gynecological symptoms. Urine was collected for the detection of schistosome eggs, and liquid chromatography diode array detection electron spray ionization mass spectrometry was used to confirm urine estrogen metabolites. **Estrogen-like metabolites thought to originate from schistosomes were confirmed in the urine of *S. haematobium*–positive patients, while negative patients had no similar metabolites. These metabolites were definitely linked to infertility.**

Unusual Parasitic Infections

Nematodes

Ghai RR, Chapman CA, Omeja PA, Davies TJ, Goldberg TL. 2014. Nodule worm infection in humans and wild primates in Uganda: cryptic species in a newly identified region of human transmission. *PLoS Negl Trop Dis* **8:**e2641. doi:10.1371/journal.pntd.0002641.

Soil-transmitted helminths continue to be a major health concern in many areas throughout the world. *Oesophagostomum* infection among humans and other primates is endemic in West Africa and has also been found in Uganda (3,000 km from identified foci in West Africa) and East Africa. These nodule worms are found in the gastrointestinal tracts of many mammals; some tend to be found in particular hosts, while others are much less host specific. Eight species of *Oesophagostomum* have been recorded in wild primates, three of which are the most common and can infect humans: *Oesophagostomum bifurcum*, *Oesophagostomum stephanostomum*, and *Oesophagostomum aculeatum*. The authors used routine and molecular methods to identify these infections. Based on these studies, it appears that ***Oesophagostomum* infection in humans may be common throughout sub-Saharan Africa, and there is great genetic diversity within this genus. There is also cross-species transmission among humans and other primates within this area.**

Eberhard ML, Ruiz-Tiben E. 2014. Cutaneous emergence of *Eustrongylides* in two persons from South Sudan. *Am J Trop Med Hyg* **90:**315–317.

Two large, viable worms were collected from the lower limbs of two individuals in South Sudan. The worms were seen as a part of the South Sudan Guinea Worm Eradication Program surveillance actions in areas thought to be at risk for Guinea worm disease (*Dracunculus medinensis*). **The worms (7 and 8 cm) were identified as fourth-stage larvae of *Eustrongylides*. This is the only case report of these worms emerging from the skin; the five earlier publications on human infection required surgery for worm removal from the peritoneal cavity.**

Cestodes

Roesel C, Welter S, Stamatis G, Theegarten D, Tappe D. 2014. Management of a chest-wall soft-tissue tumor caused by an infection with the larval tapeworm pathogen *Taenia crassiceps*. *Am J Trop Med Hyg* **91:**541–543.

A chest-wall lesion in an immunocompetent patient led to a presumptive diagnosis of a malignancy. Histopathology and PCR identified the larval stage of *Taenia crassiceps*. The tumor was surgically removed. Treatment options for immunocompromised and immunocompetent patients were reviewed. *Taenia crassiceps* causes a rare human tapeworm infection and is primarily found in the Holarctic region as an infection that circulates between canids and rodents. An increased number of cases have been reported in recent years. Of the infected patients, 47% were immunocompromised because of AIDS or hematological malignancy. Hematoma or traumatically altered or inflamed tissue appears to be a predilection site for an infection. Transmission occurs by infective eggs. No commercial serological tests are available; however, cross-reactions occur between *Taenia solium* and *T. crassiceps*. The larvae proliferate by posterior budding, making complete resections very difficult. In immunocompromised patients or patients with parasites located in the eye or brain, additional therapy with albendazole and praziquantel is recommended, as is monitoring for disease recurrence.

Trematodes

Gállego L, González-Moreno O, Gracenea M. 2014. Terrestrial edible landsnails as vectors for geographic dissemination of *Brachylaima* species. *J Parasitol* **100:**674–678.

Cornu aspersum (= syn. *Helix aspersa*) snails imported into northeast Spain from Algeria and South Africa are purchased in markets for human consumption. Samples of these snails were analyzed related to the possible geographic dissemination of *Brachylaima* species. Prevalence and viability studies of metacercariae were performed on Algerian and South African *Brachylaima* worms. Variables (16) in metacercariae and adults (18) were investigated. **Imported edible *C. aspersum* snails contribute significantly to the potential geographic spread of *Brachylaima* trematodes by harboring infective metacercariae which could easily infect animals.** Geographic spread could be enhanced by commerce in terrestrial snails such as *C. aspersum*.

Jung WT, Lee KJ, Kim HJ, Kim TH, Na BK, Sohn WM. 2014. A case of *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) infection diagnosed by colonoscopy. *Korean J Parasitol* **52:**287–290.

Echinostomiasis is a foodborne intestinal trematode infection, primarily in Southeast Asia and the Far East. Human cases in Korea and Japan have been occasionally diagnosed through worm extraction via endoscopy. Most of these infections were caused by *Echinostoma hortense*. The authors detected two live worms of *Echinostoma cinetorchis* in the ascending colon of a Korean man (68 years old) admitted with intermittent right lower quadrant abdominal pain for 5 days. During colonoscopy, one worm was found attached to the proximal ascending colon, and the other was seen on the mid-ascending colon. Both worms were removed from the mucosal surface and identified as *E. cinetorchis* from the characteristic head crown (37 collar spines including 5 end-group ones on both sides), the lack of testes, and eggs measuring 108×60 μm with abopercular wrinkles. After therapy with praziquantel, the patient's symptoms disappeared. **Although the patient had a history of eating raw beef and raw fish, the infection source in this case is probably raw frogs eaten 2 months ago. This is the first case of endoscopy-diagnosed *E. cinetorchis* infection in Korea.**

Antibody and Antigen Detection in Parasitic Infections (General Immunology) (See Also Individual Relevant Chapters)

Protozoa

Marin C, Concha-Valdez F, Canas R, Gutierrez-Sanchez R, Sanchez-Moreno M. 2014. Anti-*Trypanosoma cruzi* antibody detection in eastern Andalusia (Spain). *Trans R Soc Trop Med Hyg* **108:**165–172.

Although we tend to think of *Trypanosoma cruzi* infections as being from Latin America, they are also endemic in other areas. Currently, Spain has the highest prevalence in Europe. The authors tested eight diagnostic tests for antibody in 550 blood specimens; a range of values from 1.1 to 4.2% were obtained. The highest percentage was obtained using the ELISA detection of semi-purified superoxide dismutase excreted by *T. cruzi* (ELISA-SODe). **Based on these results, antibody detection using this particular biomarker could be used for either screening and/or confirmatory testing.**

Helminths

Mounsey K, Kearns T, Rampton M, Llewellyn S, King M, Holt D, Currie BJ, Andrews R, Nutman T, McCarthy J. 2014. Use of dried blood spots to define antibody response to the *Strongyloides stercoralis* recombinant antigen NIE. *Acta Trop* **138:**78–82.

Serologic testing for the diagnosis of *Strongyloides stercoralis* infection using the NIE antigen has proven to be very effective; the NIE antigen is a 31-kDa recombinant antigen obtained from the L3-stage larvae. **The authors report on antibody detection from dried blood spots,** primarily for improved sample collection and specimen storage requirements. Results using this approach agreed with earlier study results and confirmed the high seroprevalence of strongyloidiasis in Australian indigenous communities.

General Immunology

Protozoa

Tan JJ, Capozzoli M, Sato M, Watthanaworawit W, Ling CL, Mauduit M, Malleret B, Grüner AC, Tan R, Nosten FH, Snounou G, Rénia L, Ng LF. 2014. An integrated lab-on-chip for rapid identification and simultaneous differentiation of tropical pathogens. *PLoS Negl Trop* Dis **8:**e3043. doi:10.1371/journal.pntd.0003043.

In this study, a microfluidic lab-on-chip integrating multiplex molecular amplification and DNA microarray hybridization was developed for simultaneous detection and species differentiation of 26 globally important tropical pathogens. Test performance for each pathogen ranged from 10^2 to 10^3 DNA or RNA copies. This lab-on-chip approach was evaluated using 170 retrospective patient specimens from Singapore and Thailand with sensitivity and specificity as follows: 83.1% and 100% for *Plasmodium falciparum*; 91.3% and 99.3% for *Plasmodium vivax*; 90.0% and 100% for Chikungunya virus; and 85.0% and 100% for Dengue virus serotype 3 compared to reference methods. **These test results confirm the feasibility of an amplification microarray-based approach for detection and identification of certain tropical pathogens.**

Rodrigues V, Cordeiro-da-Silva A, Laforge M, Quaissi A, Akharid K, Silvestre R, Estaquier J. 2014. Impairment of T cell function in parasitic infections. *PLoS Negl Trop Dis* **8:**e2567.

It is well known that T cell responses are extremely important in the development of protective immunity against parasitic infections. **However, eradication of these infections is rarely accomplished, thus resulting in the subsequent development of chronic infections.** This chronic state often leads to a loss of T cell function (T cell exhaustion). There are key differences between T cell exhaustion (antigen persistence, chronic activation, loss of cytotoxic or helper activities) and T cell anergy (impaired antigen presentation, reduced costimulation). Specific organisms discussed include *Leishmania* spp., *Plasmodium* spp., *Toxoplasma gondii*, and *Trypanosoma cruzi*. These intracellular organisms also tend to inhibit T cell memory and reduce the overall number of T cells available over time.

Treatment (See Also Individual Relevant Chapters)

Thriemer K, Van Hong N, Rosanas-Urgell A, Phuc BQ, Ha DM, Pockele E, Guetens P, Van Van N, Duong TT, Amambua NA, D'Alessandro U, Erhart A. 2014. Delayed parasite clearance after treatment with dihydroartemisinin-piperaquine in *Plasmodium falciparum* malaria patients in central Vietnam. *Antimicrob Agents Chemother* **58:**7049–7055.

Decreased susceptibility of *Plasmodium falciparum* toward artemisinin-based drugs has been well documented from the Thai-Cambodian border and from the Thai-Myanmar border. The authors conducted a 42-day *in vivo* and *in vitro* efficacy study, in which treatment was monitored and blood samples were collected twice daily until the smears were negative. Also, genotyping, qPCR, and sensitivity testing were completed. Parasite clearance time was 61.7 hours; the PCR was estimated to be 100% at day 28 and 97.7% at day 42. At day 3, the *P. falciparum* prevalence by genotyping quantitative PCR was 2.5 times higher than by microscopy. **In central Vietnam, the effectiveness of dihydroartemisinin-piperaquine (DHA-PPQ) remains acceptable, but overall clearance times and rates confirm emerging artemisinin resistance.**

García-Bournissen F, Moroni S, Marson ME, Moscatelli G, Mastrantonio G, Bisio M, Cornou L, Ballering G, Altcheh J. 2015. Limited infant exposure to benznidazole through breast milk during maternal treatment for Chagas disease. *Arch Dis Child* **100:**90–94

Benznidazole (BNZ) is a safe and effective drug for the treatment of Chagas' disease in children. However, in adults it is contraindicated during breastfeeding, since BNZ transfer into breast milk has not been thoroughly investigated. BNZ was measured in plasma and milk from women and their breastfed infants on admission and on the 7th and 30th days of therapy, and then monthly for 6 months. **Limited transfer of BNZ into breast milk and normal clinical findings in the babies suggest that BNZ treatment for Chagas' disease during breast feeding is relatively risk-free for the baby.**

Liu YX, Wu W, Liang YJ, Jie ZL, Wang H, Wang W, Huang YX. 2014. New uses for old drugs: the tale of artemisinin derivatives in the elimination of *Schistosomiasis japonica* in China. *Molecules* **19:**15058–15074.

Artemisinin (qinghaosu) and its three major derivatives—artemether, artesunate, and dihydroartemisinin—have proven to be effective against all species of the malaria parasite. Since the early 1980s, these drugs have also been confirmed as being effective against *Schistosoma* spp. and have been used to prevent and treat schistosomiasis in China. **Currently, in China, praziquantel is the only available drug used for the treatment of *Schistosomiasis japonicum* infections. In the national control program, this drug has been used for more than 30 years. Although no evidence of praziquantel resistance has been detected in isolates of *S. japonicum*, resistance has been produced in the laboratory. This fact strongly suggests that *S. japonicum* may eventually develop resistance.** Although China's goal is schistosomiasis elimination, potential development of praziquantel resistance may prevent reaching this goal. Unfortunately, reduced sensitivity to artemether, artesunate, and dihydroartemisinin has also been seen in praziquantel-resistant *S. japonicum.*

Saunders DL, Vanachayangkul P, Lon C, U.S. Army Military Malaria Research Program, National Center for Parasitology, Entomology, and Malaria Control (CNM), Royal Cambodian Armed Forces. 2014. Dihydroartemisinin-piperaquine failure in Cambodia. *N Engl J Med* **37:**484–485.

Dihydroartemisinin-piperaquine, one of the only drugs effective against multidrug-resistant *Plasmodium falciparum,* was adopted as the primary antimalarial drug in Cambodia in 2010. The authors conducted a trial evaluating the effectiveness of dihydroartemisinin-piperaquine for uncomplicated *P. falciparum* malaria. Adults with parasite loads of 1,000 to 200,000 parasites per cubic millimeter were given 3 days of dihydroartemisinin-piperaquine treatment (cumulative doses, 360 mg of dihydroartemisinin and 2,880 mg of piperaquine), with 42 days of follow-up. Dihydroartemisinin-piperaquine has been effective in Africa and Southeast Asia; however, the high failure rate in this study compared with 2010 is the second report of poor clinical outcomes in Cambodia. **Increased treatment failures, parasite clearance times, and piperaquine IC_{50} values (doubled) from 2010 to 2013 indicate continued developing resistance to both drug components.**

Nagel R, Bielefeldt-Ohmann H, Traub R. 2014. Clinical pilot study: efficacy of triple antibiotic therapy in *Blastocystis* positive irritable bowel syndrome patients. *Gut Pathog* **6:**34.

***Blastocystis* spp. are the most commonly reported gut protozoa worldwide, an infection that has been linked to irritable bowel syndrome (73% versus 27% in controls).** Although many changes in the enteric and nervous system in these patients, particularly those with diarrhea-predominant **irritable bowel syndrome**, have been documented, specific changes have been linked to *Blastocystis* spp. infections. These changes include the following: activation of IL-8, increased permeability in colonic epithelial cells, and degradation of IgA (possibly from cysteine proteases). Although metronidazole has been the drug of choice, results vary from 0 to 100%, and often retreatment is required. **In the study patients (14 days of diloxanide furoate, trimethoprim-sulfamethoxazole, and secnidazole), 60% were organism-free at 6 weeks, suggesting that triple antibiotic therapy may be a good alternative to single antibiotic therapy.**

Testing of Stool Specimens

Parija SC, Mandal J, Ponnambath DK. 2014. Laboratory methods of identification of *Entamoeba histolytica* and its differentiation from look-alike *Entamoeba* spp. *Trop Parasitol* **4:**90–95.

Entamoeba histolytica causes intestinal and extraintestinal amebiasis and is a common parasitic cause of morbidity and mortality in many countries. Detection and differentiation of pathogenic *E. histolytica* from nonpathogenic *Entamoeba* spp. (*Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba bangladeshi*) are important in patient management. Most diagnostic tests currently in use fail to differentiate among the *Entamoeba* spp. Molecular-based methods are more sensitive, easy to perform, more widely used, and can differentiate the various species. DNA microarray technology has been found to be another promising tool for the diagnostic and epidemiological evaluation of amebiasis.

Stark D, Roberts T, Ellis JT, Marriott D, Harkness J. 2014. Evaluation of the EasyScreen™ enteric parasite detection kit for the detection of *Blastocystis* spp., *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba* complex, and *Giardia intestinalis* from clinical stool samples. *Diagn Microbiol Infect Dis* **78:**149–152.

The authors evaluated the EasyScreen Enteric Parasite Detection Kit (Genetic Signatures, Sydney, Australia) for the detection and identification of five enteric human parasites on 358 fecal specimens: *Blastocystis* spp., *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba* complex, and *Giardia intestinalis*. Compared with PCR and microscopy, the EasyScreen Enteric Parasite Detection Kit resulted in 92 to 100% sensitivity and 100% specificity. No cross-reactivity with other bacterial, viral, and/or protozoan species was seen. The EasyScreen Kit does not detect *Cystoisospora belli* or *Cyclospora cayetanensis*, and it does not differentiate between pathogenic and nonpathogenic *Entamoeba* spp.

APPENDIX 8

Molecular Panels for Parasitology

Reagents contain ingredients that could be irritating or caustic if they come in contact with skin, eyes, or mucous membranes. Wear gloves, safety glasses, and laboratory coat, and use standard laboratory precautions when handling. If swallowed, call a physician. In case of skin or eye contact, flush with copious amounts of water. *Pathogenic microorganisms including hepatitis viruses and human immunodeficiency virus may be present in clinical specimens*. Proper handling and disposal methods should be established. Wipe up spillage of patient specimens immediately and disinfect with an appropriate disinfectant. Treat the cleaning materials as biohazardous waste.

The test options listed below are examples of Food and Drug Administration (FDA)-approved molecular diagnostic tests for parasites. In the future, we expect to see more test options, since many test parasite panels are currently under development.

A. APTIMA *Trichomonas vaginalis* Assay

Although there are many molecular methods for identifying parasites published in the literature, most of these tests were developed in-house and have not been approved by the FDA. However, there are several FDA-approved molecular diagnostic tests for parasites, including the APTIMA *Trichomonas vaginalis* Assay (GenProbe, San Diego, CA). The APTIMA *Trichomonas vaginalis* Assay is a nucleic acid test that utilizes target capture, transcription-mediated amplification (TMA), and hybridization protection assay (HPA) technologies that have previously been used for detection of *Neisseria gonorrheae* and *Chlamydia trachomatis*. It also accepts the same specimen types (i.e., clinician-collected vaginal and endocervical swabs, female urine, and PreservCyt solution). However, urine, vaginal swab, and PreservCyt Solution liquid Pap specimen sampling are not designed to replace cervical exams and endocervical specimens for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents. Reliable results depend on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary.

The APTIMA assay provides high sensitivity and specificity and does not require organism viability or motility. There are approximately 7 million new cases of *T. vaginalis* infection in the United States per year (1). This figure probably represents an underestimate of the disease burden, considering that there are no recommended screening guidelines and the most commonly used testing methods such as wet mounts and culture are insensitive. Clinical infection is associated with cervical neoplasia, cervical carcinoma, tubal infertility, posthysterectomy infection, pelvic inflammatory disease, preterm rupture of membranes, preterm birth, low birth weight infants, neonatal infection, and increased transmission of HIV (2). It has been estimated that 10 to 50% of *T. vaginalis* infections in women are asymptomatic, and in men the proportion may even be higher (3–7). It is important to remember that therapeutic failure or success cannot be determined with the APTIMA *Trichomonas vaginalis* Assay since nucleic acid may persist following appropriate antimicrobial therapy.

KEY POINTS

1. A negative result does not exclude a possible infection since results depend on adequate specimen collection. Test results may be affected by improper specimen collection, technical error, specimen mix-up, or target levels below the assay limit of detection.
2. A negative result does not exclude a possible infection because the presence of *Trichomonas tenax* or *Pentatrichomonas hominis* in a specimen may affect the ability to detect *T. vaginalis* rRNA. However, these organisms are relatively rare and may not present a problem. Also, performance of the APTIMA *Trichomonas vaginalis* Assay has not been evaluated in the presence of *Dientamoeba fragilis*.
3. The APTIMA *Trichomonas vaginalis* Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the number of organisms in a specimen.
4. If a specimen contains few *T. vaginalis* organisms, uneven distribution of the trichomonads may occur, which may affect the ability to detect *T. vaginalis* rRNA in the collected material. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary.
5. This test has not been FDA cleared for male clinical specimens.

B. Affirm VPIII Microbial Identification Test

The Affirm VPIII DNA probe technology (Becton Dickinson and Co, Franklin Lakes, NJ) provides a dependable, rapid means for the differential detection and identification of three organisms causing vaginitis: *Candida* species, *Gardnerella vaginalis*, and *T. vaginalis* (8, 9). The test features an easy-to-read color reaction that is more accurate than current microscopic methods such as wet mounts and culture for the detection of the causative agents of vaginitis. Also, the test performance is not affected by "difficult specimens" (collected during menstruation, after intercourse), self-medication, or the presence of mixed infections.

The test uses two distinct single-stranded nucleic acid probes for each organism, a capture probe and a color development probe, that are complementary to unique genetic sequences of the target organisms. The capture probes are immobilized on a bead embedded in a probe analysis card (PAC), which contains a separate bead for each target organism. The color development probes are contained in a multiwell reagent cassette. During the procedure, the assay aligns complementary nucleic acid strands to form specific, double-stranded complexes called hybrids. For each of the three organisms, this hybrid is composed of capture and color development single-stranded nucleic acid probes, complementary to the released target nucleic acid analyte. Enzyme conjugate then binds to the captured analyte, thus providing the test results. A positive result is a visible blue color reaction on the organism-specific bead embedded in the Affirm PAC. Each PAC contains three test beads, one for each organism, plus positive and negative control beads to ensure test quality. The total time to results is under 45 min, with less than 2 min of hands-on time needed. The simple, automated procedure can be performed with minimal training.

KEY POINTS

1. When using the Affirm VPIII Ambient Temperature Transport System (ATTS): The total time *between sample collection and proceeding with sample preparation should be no longer than* 72 h when the specimen is stored at ambient conditions (15 to 30°C). The system has also been qualified for transport use at refrigerated conditions (2 to 8°C).
2. The Affirm VPIII Microbial Identification Test includes two internal controls on each PAC: a positive control bead and a negative control bead. These control beads are tested simultaneously with each patient specimen, ensuring the proper performance of the PAC, reagent cassette, and processor. The positive control also ensures the absence of specimen interference. The negative control also ensures the absence of nonspecific binding from the specimen.
3. The assay is intended to be used with the Affirm VPIII Ambient Temperature Transport System, the Affirm VPIII Sample Collection Set, or the swabs provided in the Affirm VPIII Microbial Identification Kit. Other methods of collection have not been evaluated. Optimal test results require appropriate specimen collection. Test results may be affected by improper specimen collection, handling, and/or storage conditions. A negative test result does not exclude the possibility of vaginitis/vaginosis.
4. Vaginitis/vaginosis is most frequently caused by *G. vaginalis*, *Candida* species, and *T. vaginalis*. Vaginitis symptoms may also be seen in toxic shock syndrome (caused by *Staphylococcus aureus*) or may be caused by nonspecific factors or by specific organisms. Mixed infections may occur. Therefore, a test indicating the presence of *Candida* species, *G. vaginalis*, and/or *T. vaginalis* does not rule out the presence of other organisms. Bacterial vaginosis is not caused by a single organism, but is thought to reflect a shift in the vaginal flora.
5. The performance of this test on patient specimens collected during or immediately after antimicrobial therapy is unknown. The presence or absence of *Candida* species, *G. vaginalis*, or *T. vaginalis* cannot be used as a test for therapeutic success or failure.

C. BD MAX Enteric Parasite Panel

The BD MAX Enteric Parasite Panel (Becton, Dickinson and Company, Sparks, MD) is a multiplex qualitative *in vitro* diagnostic test that simultaneously and differentially detects DNA from *Giardia lamblia*, *Cryptosporidium* (*Cryptosporidium hominis* and *Cryptosporidium parvum*), and *Entamoeba histolytica* in unpreserved and preserved (10% formalin) stool specimens (10–12). These pathogens represent commonly isolated and highly pathogenic organisms. Results from this assay are available in approximately 4 hours, require minimal technologist time, and may replace traditional methods such as microscopy and immunoassay.

Positive results do not rule out coinfection with other organisms that are not detected by this test and may not be the sole cause of patient illness. Negative results in the setting of clinical illness compatible with gastroenteritis and/or colitis may be due to infection by pathogens that are not detected by this test or noninfectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

A soft to diarrheal stool is collected, homogenized, and looped into a sample buffer tube (SBT). The SBT is closed with a septum cap and heated on the BD prewarm heater to lyse the parasite organisms. The SBT is then vortexed and transferred to the BD MAX system. The cells are lysed, nucleic acids are extracted on magnetic beads and concentrated, and then an aliquot of the eluted nucleic acids is added to PCR reagents which contain the target-specific primers used to amplify the genetic targets. The assay also includes a sample processing control (SPC), which is present in the extraction tube and undergoes the extraction, concentration, and amplification steps to monitor for inhibitory substances or instrument or reagent failure. The BD MAX system automates sample lysis, nucleic acid extraction and concentration, reagent rehydration, nucleic acid amplification, and detection of the target nucleic acid sequence using real-time PCR. Amplified targets are detected with hydrolysis probes labeled with quenched fluorophores. The amplification, detection, and interpretation of the signals are done automatically by the BD MAX system.

KEY POINTS

1. To avoid amplicon contamination, do not break apart the BD MAX PCR cartridges after use. The seals of the BD MAX PCR cartridges are designed to prevent contamination.
2. In cases where open-tube PCR tests are conducted in the laboratory, care must be taken to ensure that the BD MAX Enteric Parasite Panel, any additional reagents required for testing, and the BD MAX system are not contaminated. Gloves must be changed before manipulating reagents and cartridges.
3. BD MAX Enteric Parasite Panel components are stable at 2 to 25°C through the stated expiration date. Do not use expired components.
4. A BD MAX Enteric Parasite Panel positive result does not necessarily indicate the presence of viable organisms. It indicates the presence of DNA from *E. histolytica*, *G. lamblia*, *C. parvum*, or *C. hominis*, allowing for identification of the BD MAX Enteric Parasite Panel organisms.
5. As with all PCR-based *in vitro* diagnostic tests, extremely low levels of target below the analytical sensitivity of the assay may be detected, but results may not be reproducible. False negatives may also occur if the level of organism nucleic acid is below the threshold of detection.
6. False negative results may occur due to loss of nucleic acid from inadequate collection, transport, or storage or due to inadequate organism lysis. The SPC has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The SPC does not indicate if nucleic acid has been lost due to inadequate collection, transport, or storage or whether cells have been inadequately lysed.
7. BD MAX Enteric Parasite Panel results may or may not be affected by concurrent antimicrobial therapy, which may reduce the amount of target present.
8. The performance of this test has not been established for monitoring treatment of *G. lamblia*, *C. hominis*, *C. parvum*, or *E. histolytica*. This test is a qualitative test and does not provide quantitative values or indicate the quantity of organisms present.
9. The performance of this test has not been evaluated for immunocompromised individuals or for patients without symptoms of gastrointestinal infection.
10. The effect of interfering substances has only been evaluated for those listed in this labeling. Potential interference has not been evaluated for substances other than those described in the "Interference" section.
11. Cross-reactivity with organisms other than those listed in the "Analytical Specificity" section of the package insert has not been evaluated.

D. BioFire FilmArray Gastrointestinal Panel

The FilmArray Gastrointestinal Panel (BioMérieux, Marcy l'Etoile, France) is designed for the simultaneous detection of 22 enteric pathogens directly from stool specimens: *Campylobacter* spp. (*Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter upsaliensis*), *Clostridium difficile* (toxin A/B), *Plesiomonas shigelloides*, *Salmonella*, *Vibrio* spp. (*Vibrio Parahaemolyticus*, *Vibrio vulnificus*, *Vibrio cholerae*), *Yersinia enterocolitica*, enteroaggregative *Escherichia coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, Shiga-like toxin-producing *E. coli* (*stx1/2*), STEC O157, *Shigella*/enteroinvasive *E. coli*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *E. histolytica*, *G. lamblia* (*Giardia intestinalis*, *Giardia duodenalis*) Adenovirus F 40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, and Sapovirus (Genogroups I, II, IV, and V) (13–15). This test is a qualitative test and does not provide a quantitative value for the organism(s) in the sample.

The FilmArray GI pouch is a closed, disposable system that houses all the chemistry required to isolate, amplify, and detect nucleic acid from multiple gastrointestinal pathogens within a single stool specimen. The rigid plastic component (fitment) of the FilmArray GI pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user of the FilmArray GI Panel loads the sample into the FilmArray GI pouch, places the pouch into the FilmArray instrument, and starts the run. All other operations are automated.

The sample volume requirement is 200 µl. The FilmArray GI Panel test consists of automated nucleic acid extraction, reverse transcription, amplification, and analysis, with results available in 1 hour per run (i.e., per specimen). Each FilmArray pouch contains an internal nucleic acid extraction control and a PCR control. FilmArray GI Panel runs are considered valid if the run completes normally and internal controls are passed. The FilmArray software performs automated result analysis with each target in a valid run reported as "Detected" or "Not Detected." If either internal control fails, the software automatically provides a result of "Invalid" for all panel analytes.

KEY POINTS

1. Stool samples may contain a high concentration of organisms. To avoid possible contamination, samples should be processed in a biosafety cabinet. If a biosafety cabinet is not used, a dead air box, a splash shield, or a face shield should be used when preparing samples. A biosafety cabinet or work station that is used for performing stool pathogen testing (e.g., culture, enzyme immunoassay) should not be used for sample preparation or pouch loading.

(continued)

KEY POINTS *(continued)*

2. Prior to processing a sample, thoroughly clean both the work area and the FilmArray pouch loading station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue build-up and potential PCR inhibition, wipe disinfected surfaces with water. Samples and pouches should be handled one at a time. Change gloves and clean the work area between each sample.
3. Stool specimens should be collected in Cary Blair transport media according to the manufacturer's instructions. A 200-µl sample is required for testing. Specimens should be processed and tested as soon as possible, though they may be stored at room temperature or under refrigeration for up to 4 days.
4. The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The freeze-dried yeast is present in the pouch and becomes rehydrated when the sample is loaded. The control material is carried through lysis, nucleic acid purification, reverse transcription, first-stage PCR, dilution, second-stage PCR, and DNA melting. A positive control result indicates that all steps carried out in the FilmArray GI pouch were successful.
5. The PCR2 control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that second-stage PCR was successful.
6. Good laboratory practice recommends running external positive and negative controls regularly. Enteric transport media can be used as an external negative control. Previously characterized positive stool samples or negative samples spiked with well-characterized organisms can be used as external positive controls. External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable.
7. Once melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the melting temperature for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.
8. If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
9. The FilmArray GI Panel contains two assays (Crypt 1 and Crypt 2) for detection of *Cryptosporidium* spp. Detection of approximately 23 *Cryptosporidium* spp. occurs, including the most common species of human clinical relevance (i.e., *C. hominis* and *C. parvum*), as well as several less common species (e.g., *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium cuniculus*, *Cryptosporidium muris*, and *Cryptosporidium suis*). The assays do not differentiate between *Cryptosporidium* spp. and the very rare species *Cryptosporidium bovis*, *Cryptosporidium ryanae*, and *Cryptosporidium xiaoi*, which may not be detected. A positive result for either or both assays will give a "*Cryptosporidium* Detected" test result.
10. The FilmArray GI Panel contains a single assay (Ehist) for the detection of *E. histolytica*, the only *Entamoeba* species implicated in gastroenteritis. This assay may cross-react with the closely related *Entamoeba dispar* when present at higher levels (approximately 10^5 organisms/ml or greater).
11. The "Result Summary" section of the test report lists the result for each target tested by the panel. Possible results for each organism are "Detected," "Not Detected," "Not Applicable (N/A)," or "Invalid." See the "Results Summary" section in the instruction booklet for detailed information about interpretation of test results and appropriate follow-up for "Invalid" results.
12. The performance of this test has only been validated with human stool collected in Cary Blair transport medium following the media manufacturer's instructions. It has not been validated for use with other stool transport media, raw stool, rectal swabs, endoscopy stool aspirates, or vomitus. Test performance has not been established for patients without signs and symptoms of gastrointestinal illness.
13. Virus, bacteria, and parasite nucleic acid may persist *in vivo* independent of organism viability. Additionally, some organisms may be carried asymptomatically. Detection of organism targets does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms. The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.

E. Luminex

In January 2013, the FDA approved the first test that can simultaneously detect 11 common viral, bacterial, and parasitic (*Cryptosporidium*, *Giardia*) causes of infectious gastroenteritis from a single patient fecal specimen (xTAG Gastrointestinal Pathogen Panel [GPP], Luminex, Inc., Austin, TX) (14, 16–20). The xTAG GPP is a multiplexed nucleic acid test for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis.

The target is amplified using PCR or reverse transcription PCR, then analyzed with Luminex xTAG technology to detect the presence or absence of each pathogen in the panel. The test detects 11 gastrointestinal pathogen targets: *Campylobacter* (*C. jejuni*, *C. coli*, and *C. lari* only), *Clostridium difficile* toxin A/B, *Cryptosporidium* (*C. parvum* and *C. hominis* only), *E. coli* O157, enterotoxigenic *E. coli* (ETEC) LT/ST, *Giardia* (*G. lamblia* only, also known as *G. intestinalis* and *G. duodenalis*), Norovirus GI/GII, Rotavirus A, *Salmonella*, Shiga toxin-producing *E. coli* (STEC) stx1/stx2, and *Shigella* (*Shigella boydii*, *Shigella sonnei*, *Shigella flexneri*, and *Shigella dysenteriae*). The xTAG GPP is capable of delivering multiple results within 5 hours. The assay is cleared on the widely available Luminex 100/200 system. Additionally, simultaneous molecular testing on a single sample within a single shift provides significant benefit to laboratories in terms of workflow and resource utilization.

Human stool samples are pretreated and then subjected to nucleic acid extraction. For each sample, 10 µl of extracted nucleic acid is amplified in a single multiplex RT-PCR/PCR reaction. Each target or internal control in the sample results in PCR amplimers ranging from 58 to 202 bp (not including the 24-mer tag). A 5-µl aliquot of the RT-PCR product is then added to a hybridization/detection reaction containing bead populations coupled to sequences from the Universal Array ("antitags"), streptavidin, R-phycoerythrin conjugate. Each Luminex bead population detects a specific microbial target or control through a specific tag/anti-tag hybridization reaction. Following the incubation of the RT-PCR products with the xTAG GPP Bead Mix and xTAG Reporter Buffer, the Luminex instrument sorts and reads the hybridization/detection reactions. A signal or median fluorescence intensity is generated for each bead population. These fluorescence values are analyzed to establish the presence or absence of bacterial, viral, or parasitic targets and/or controls in each sample. A single multiplex reaction identifies all targets.

KEY POINTS

1. Due to the open system design of the platform, there is a potential for contamination. The "Intended Use" (package insert) of this device states that all positive results using the xTAG GPP assay are presumptive and need to be confirmed by another FDA-cleared or approved assay or acceptable reference method. All results should be used and interpreted in the context of a full clinical evaluation as an aid in the diagnosis of gastrointestinal infection. Confirmed positive results do not rule out coinfection with other organisms that are not detected by this test and may not be the sole or definitive cause of patient illness. Negative xTAG GPP results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or noninfectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.
2. Analyte targets (virus, bacteria, or parasite nucleic acid sequences) may persist *in vivo*, independent of virus, bacteria, or parasite viability. Detection of analyte target(s) does not guarantee that the corresponding live organism(s) is present or that the corresponding organism(s) is the causative agent for clinical symptoms.
3. *Cryptosporidium*: the xTAG GPP assay detects *C. parvum* and *C. hominis* only.
4. *Giardia*: the xTAG GPP assay detects *G. lamblia* only (also known as *G. intestinalis* and *G. duodenalis*).
5. There is a risk of false-negative values due to the presence of strain/species sequence variability in the targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
6. The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
7. Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely prevalent when disease is high. False-positive test results are more likely during periods when prevalence is low.
8. This test is a qualitative test and does not provide the quantitative value of detected organism present.
9. Known strains or positive clinical samples for the targeted viruses, bacteria, or parasites should be included in routine quality control procedures ("external controls") as positive controls for the assay. One or more of these external controls are analyte positive controls and should be included with each batch of patient specimens. Controls positive for different targets should be rotated from batch to batch. External controls should be prepared, extracted, and tested in the same manner as patient samples (http://www.accessdata.fda.gov/cdrh_docs/reviews/K121454.pdf).
10. Failure to correctly interpret test results in the context of other clinical and laboratory findings may lead to inappropriate or delayed treatment. For example, a microorganism present as a colonizer may be correctly detected but not be the true cause of illness. Although this identical risk would be present from the use of any microbiological assay in this setting, simultaneous testing of multiple analytes in a multiplex assay may be more likely to detect an unanticipated colonizer that might not be tested for individually.
11. It should be recognized that the device is intended for use as an aid in the diagnosis of gastroenteritis in conjunction with clinical presentation and the results of other laboratory tests. Both clinical presentation and other results would likely substantially mitigate concerns with both false-positive and false-negative test results.

F. Other Pending Molecular Tests

Several other molecular tests are in clinical trials for the detection of select gastrointestinal parasites. These tests are molecular gastrointestinal panels and target the most commonly occurring parasitic stool pathogens. Although there are laboratory-developed tests for most parasites, these are not commercially available or are available only in specialized testing centers. When such tests are used, attention should be given to the use of internal amplification controls to detect inhibition, since common specimens, such as blood and stool, contain PCR inhibitors. Thorough validation is required before these are implemented for clinical testing. Specific organisms under consideration include *E. histolytica*, *Blastocystis hominis* (*Blastocystis* spp.), and *D. fragilis*. Blood parasites include *Plasmodium* spp. (including *Plasmodium knowlesi*), *Trypanosoma* spp., and *Leishmania* spp.

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APPENDIX 9

Frequently Asked Questions about Diagnostic Parasitology

As laboratory personnel become more widely cross-trained, the availability of people who have expertise in diagnostic parasitology will become more limited. Over the years, many questions have been asked regarding various aspects of diagnostic medical parasitology. Answers to these questions may be helpful for those working in this area of microbiology and may provide some "tips of the trade" that are learned through many years of bench experience.

FECAL SPECIMENS

SPECIMEN COLLECTION (1–4; this volume)

1. What has been the "gold standard" for stool collection systems?

The standard two-vial collection set has been used for many years and consists of one vial of 10% formalin from which the concentration is performed and one vial of liquid fixative (base fixative can be mercury [Schaudinn's fixative] or nonmercury to which has been added the plastic resin powder polyvinyl alcohol, PVA [stool adhesive]) from which the permanent stained smear is prepared.

2. What is the basis for the recommendation that three stools should be collected on alternate days, rather than 3 days in a row or three in one day?

Intestinal protozoa are shed in the stool on a cyclic basis. It is generally accepted that this time frame is approximately 10 days, so if specimens are collected too close together, sampling may occur at a low point in the cycle and the organisms may be missed. By collecting specimens over a wider time frame, days with heavier shedding may be sampled as well.

3. Can the laboratory accept three stools collected 3 days in a row?

Yes, but chances of sampling during the total time frame for shedding (10 days) might be more likely to include days of heavy shedding.

4. Is it ever appropriate to accept three stools in one day from the same patient?

Generally, the answer is no, with rare exceptions. If a patient has severe diarrhea or dysentery, there is a large dilution factor that may make finding any organisms present more difficult. Therefore, after consultation with the physician, more than one stool could be examined within any one day.

5. Is it good laboratory practice to accept two (rather than three) stools for the ova and parasite (O&P) examination?

Although the examination of three stool specimens provides a more statistically accurate result, many laboratories feel the percentage of organism recovery with the examination of two stools is acceptable. Percent recovery varies from ~80% to over 95% with the submission and examination of two stool specimens from a single patient. The examination of a single stool specimen is only about 50% accurate in the detection of parasites, especially intestinal protozoa. One study (5) found the following increases when examining the third stool:

- Yield increased 22.7%: *Entamoeba histolytica*
- Yield increased 11.3%: *Giardia lamblia*
- Yield increased 31.1%: *Dientamoeba fragilis*

6. Do you have to collect the stool specimen any differently if you are looking for the coccidia (*Cryptosporidium* spp., *Cyclospora cayetanensis*, or *Cystoisospora* [*Isospora*] *belli*)?

Fresh stool or specimens preserved in routine stool fixatives (formalin- or nonformalin-based) can be used for diagnostic procedures for the identification of the coccidial oocysts (special modified acid-fast stains for the coccidia or fecal immunoassays for *Cryptosporidium* spp.). Some of the single-vial collection options (Universal Fixatives, e.g., TOTAL-FIX) are also acceptable.

7. Do you have to collect the stool specimen any differently if you are looking for microsporidia?

Fresh stool or specimens preserved in routine stool fixatives (formalin- or nonformalin-based) can be used for diagnostic procedures (modified trichrome stains) for the identification of the microsporidial spores. Some of the single-vial collection options (Universal Fixatives) are also acceptable.

FIXATIVES AND ADHESIVES

1. What are the pros and cons of using stool fixatives?

Although it might be helpful to see motile organisms in the direct saline wet preparation, often the trophozoite forms have already begun to disintegrate by the time the stool specimen reaches the laboratory. **Trophozoites DO NOT ENCYST after the stool has left the body.** To reduce the lag time between passage of the specimen and submission/arrival in the laboratory, stool fixatives are recommended. However, if only preserved stool is submitted, immunoassays that require fresh or frozen stool (e.g., *E. histolytica* Ag, *E. histolytica/Entamoeba dispar* Ag, or Triage cartridge) cannot be performed. **The benefits of fixation and preservation of organism morphology far outweigh the benefits of receiving a fresh, unpreserved stool.**

2. When selecting a single-vial stool fixative, what questions should be asked?

Can one perform a concentration, permanent stained smear, special stains for the coccidia and microsporidia, fecal immunoassay procedures, and/or PCR from the specimen received in that vial (Universal Fixative)? What stains work best with that particular fixative? Another issue for consideration involves the types and numbers of parasites that might be missed using this approach.

3. What are the advantages and disadvantages of using 5% versus 10% formalin?

Although 5% formalin is thought to provide a more "gentle" fixation of protozoa, this concentration of formalin will not always kill all helminth eggs. The 10% formalin will kill most helminth eggs (exception: *Ascaris lumbricoides, Toxocara, Baylisascaris*) but may be more damaging to protozoa. Most people probably can't tell the difference by examining clinical specimens preserved in either 5% or 10% formalin, with the exception of helminth egg viability (containing motile larva—*Ascaris, Toxocara*).

4. What is the difference between buffered and nonbuffered formalin?

Buffered formalin tends to produce fewer osmotic changes in the organisms during fixation; however, on a day-to-day basis, it may be difficult to detect morphologic differences from buffered or unbuffered formalin.

5. Should the laboratory be concerned about the amount of formalin used in the parasitology lab? (Also see a complete discussion in the "Concentrations" section.)

The amount of formalin used in diagnostic parasitology testing is minimal. The regulations governing formalin use were developed for industry, where large amounts of formalin are sometimes used. However, the regulations do indicate that any facility using formalin must be monitored. Once you have been monitored and results do not exceed the stated limits and your records are on file, there is no need to be rechecked (unless something dramatically changes in terms of your formalin volume use). **We have never heard of any microbiology laboratory coming even close to the limits, so formalin use in diagnostic parasitology is perfectly acceptable.**

6. What is PVA (stool adhesive)?

PVA stands for polyvinyl alcohol, a plastic powder/resin that is incorporated into the liquid fixative (Schaudinn's or other fixatives) and serves as an adhesive to "glue" the stool material onto the slide. **PVA itself has no preservation capability and is inert in terms of fixation.**

7. What is albumin (stool adhesive)?

Egg albumin has been used as a stool adhesive, particularly when using sodium acetate-acetic acid-formalin (SAF) that does not contain PVA plastic resin (also an adhesive). However, with the development of Universal Fixatives, smears can be prepared without using PVA or albumin.

8. What are some good stool fixative/stain combinations?

The "gold standard" has been 1 vial of 10% formalin, from which the concentration is performed. The second vial has been with mercuric-chloride liquid fixative to which has been added PVA, from which the permanent stained smear is prepared and then stained (trichrome or iron hematoxylin). Other options in terms of overall quality are:

a. TOTAL-FIX and trichrome stain
b. SAF and iron hematoxylin stain
c. UNIFIX or Z-PV and trichrome stain
d. ECOFIX and ECOSTAIN (Meridian Bioscience)
e. SAF and trichrome stain (not as good as SAF and iron hematoxylin)

9. What are Universal Fixatives?

Examples of "Universal Fixatives" are TOTAL-FIX (no mercury, no PVA, no formalin), SAF (no mercury, no PVA; *contains formalin*), and ECOFIX (no mercury, no formalin; *contains PVA*). **Currently, TOTAL-FIX is the only fixative that contains no formalin, no PVA, and no mercury. TOTAL-FIX can be used without the addition of the PVA adhesive to the fixative; adequate drying time for smears prior to staining is the most important step (minimum of 30 min to 1 h in 37°C incubator—requires more time for thicker fecal smears).**

SPECIMEN PROCESSING (1–4; this volume)

Ova and Parasite Examination

1. What procedures constitute the O&P examination?

The direct wet mount, concentration, and permanent stained smear constitute the routine O&P examination on fresh stool specimens. **If the specimens are submitted to the laboratory in stool preservatives, then the concentration and permanent stained smear should be performed; the direct wet mount is not required, since no motility will be visible.** If a laboratory indicates that it provides an O&P examination, the CAP checklist indicates that the O&P examination must include the concentration and permanent stained smear. Also, the CAP checklist requires that the direct wet smear be performed on **fresh** liquid or soft stool only (looking for motile trophozoites). There is no need to perform a direct wet mount on fresh formed stool; the possibility of seeing motile trophozoites is rare, since formed stool tends to contain only the cyst forms.

2. Why do you need to perform a permanent stained smear examination on every stool submitted for an O&P examination?

There are data to indicate that intestinal protozoa may or may not always be seen and identified from the concentration examination. **Since the permanent stained smear is designed to facilitate identification and/or confirmation of the intestinal protozoa, it is important that this procedure be performed on all stools for which the O&P has been ordered.** Also, since trophozoite stages will not be visible on the concentration examination (formalin-based fixatives; however, if present, trophozoites are often visible in concentration sediments prepared from the nonformalin-based fixatives [single-vial systems]), it is even more important to examine the permanent stained smear. Even if organisms (trophozoites and/or cysts) are seen in the concentration wet mount, they might not be identified accurately and will require confirmation from the examination of the permanent stained smear. Remember, patients who are symptomatic with diarrhea are more likely to have protozoan trophozoites in the stool, not the more resistant cyst form. This approach is consistent with the O&P examination (CAP checklist for laboratory inspections).

3. Why do you need to pour out some PVA onto paper towels prior to preparation of slides for permanent staining?

The reason this step has been included in processing directions is the following: some people were taking the PVA/stool right out of the vial and onto the slide; too much PVA was being carried over onto the slide. It takes quite a bit of time for PVA to dry, so the material often falls off during staining because the thick PVA is not yet dry. **Also, the amount of PVA (plastic powder) it takes to glue the stool onto the slide is extremely small.** So if you eliminate the excess PVA prior to making the smears, your slides will require less drying time, the stool will adhere to the glass, and you will get a better stained smear. However, if the material (PVA/stool mixture) is taken right out of the vial onto the glass slide and this approach is working (no excess PVA), there is no need to change protocols. Note: If TOTAL-FIX is being used, no PVA is used; however, drying time prior to staining is still very important.

4. Can you use concentrated sediment to prepare slides for permanent staining?

The main thing to remember is that **a routine permanent stain (e.g., trichrome stain) cannot be performed from the concentration sediment if the specimen was originally preserved in formalin or has been rinsed using formalin, saline, or water.** One can perform a trichrome stain on SAF-preserved material, but not if the specimen has been rinsed with formalin, saline, or water. If you centrifuge the fecal/fixative material (without adding any rinse reagents), then providing your fixative is compatible with the permanent stain you are using, you can use some of the sediment for smear preparation prior to staining. However, once you continue with the rinse steps, the final concentration sediment will often (again depending on the rinse fluids) not be compatible with trichrome staining. **If you want to use a single-vial fixative system (TOTAL-FIX), then you can spin down the stool/fixative mixture (using no additional rinse reagents), prepare your slide for permanent staining from the sediment, and then proceed with the regular rinses called for in the concentration procedure.**

DIAGNOSTIC METHODS (1–4; this volume)

Direct Wet Examinations

1. What is the purpose of the direct wet examination (saline versus iodine)?

This procedure is designed to allow the viewer to detect motile trophozoites (using saline); this procedure should not be performed on preserved specimens and should be reserved for fresh stool specimens that are very soft or liquid. Often, the organism identification will be presumptive; permanent stained smears will also need to be examined. If iodine is added to the direct wet mount, organism morphology may be more easily seen, but the organisms will be killed, and thus no motility will be visible.

2. How should the direct wet preparation be examined?

The entire coverslip preparation (22 × 22 mm) should be examined under low magnification (×100, 10× objective); approximately 1/3 to 1/2 of the coverslip preparation should be examined under high dry magnification (×400, 40× objective). **It is not practical to examine this preparation using oil immersion magnification (e.g., ×1,000, 100× or ×600, 60× oil immersion objectives).** Saline and/or iodine mounts can be examined; however, iodine will kill any organisms present, so trophozoite motility will no longer be visible.

3. What do you expect to see during a direct wet preparation examination?

Helminth eggs and/or larvae can be seen, as well as some protozoan cysts, trophozoites (primarily in fresh direct wet preparations from soft or liquid stool), white blood cells (WBCs), some yeast, and fecal debris. Many of the intestinal protozoa will need to be confirmed using the oil immersion magnification (×1,000, 100× oil immersion objective) for the permanent stained smear.

Concentrations

1. What is the recommended time and speed for centrifugation for the concentration method? Why is this important?

The current recommendation is for every centrifugation step in the concentration method (sedimentation) to be performed for **10 min at 500 × *g*.** If this recommendation is not followed, then small coccidian oocysts and microsporidial spores may not be recovered in the concentrate sediment. The number of organisms is greatly increased over taking the sample from the unspun specimen.

2. What is the purpose of the concentration procedure?

The purpose of the concentration is to concentrate the parasites present, either through sedimentation or flotation. The concentration is specifically designed to allow recovery of protozoan cysts, coccidian oocysts, microsporidian spores, and helminth eggs and larvae.

3. Why is the flotation concentration used less frequently than the sedimentation concentration?

There are several reasons. First, **not all parasites will float; therefore, you need to examine both the surface film and the sediment before indicating that the concentration examination is negative.** Second, the organisms must not be left in contact with the high specific gravity zinc sulfate for too long or protozoa will tend to become distorted, so the timing of the examination is more critical. Also, the specific gravity of the fluid will need to be checked periodically.

4. What specific gravity of zinc sulfate should be used for the flotation concentration procedure?

If the concentration is being performed on fresh stool, the specific gravity of the solution should be 1.18. However, if the concentration is being performed on stool preserved in a formalin-based fixative, the specific gravity of the zinc sulfate should be 1.20.

5. How should the concentration wet preparation be examined?

Formalin-ethyl acetate (substitute for ether) sedimentation concentration is the most commonly used concentration procedure. Zinc sulfate flotation will not detect operculated or heavy eggs (e.g., unfertilized *Ascaris* eggs, operculated cestode, and/or trematode eggs); both the surface film and sediment need to be examined before reporting a negative result if the flotation method is used. Smears prepared from concentrated stool are normally examined as for the direct wet mount using the low power objective (10×) and the high dry power objective (40×); use of the oil immersion objective (100×) is not recommended (organism morphology is not that clear). The addition of too much iodine may obscure helminth eggs (will mimic debris).

6. What semiautomated methods are available to read the concentration sediments?

The traditional sampling approach using pipettes and the preparation of wet smears using glass slides and coverslips can be replaced with a semiautomated sampling and viewing system from FE-5 (Apacor Ltd., Brooklyn, NY). The specimen is drawn through tubing from the mixed concentrated stool sediment into two viewing chambers that fit onto the microscope stage. The quality of the glass is excellent, and organism morphology can be easily seen within the viewing chambers. Selection of such a system often depends on the laboratory work load.

7. Are there any tips for specimen processing for detection of the microsporidia?

When early comparisons of methods were performed, the authors used a slower centrifugation compared with using unspun material. Using these methods, they felt that using unspun material was better for recovery of microsporidial spores. **However, at UCLA when we looked carefully at this approach compared with the 500 × *g* for 10 min (standard centrifugation time and speed), we found considerably more spores in the sediment.** If the stool contains a lot of mucus or is runny, just add formalin and centrifuge. Don't bother with ethyl acetate since it may pull much of the material you want to examine up into the mucus layer. If the stool is not particularly watery or doesn't contain a lot of mucus, you can treat it just like a regular concentration (but remember to make every spin at 500 × *g* for 10 min). Also, the more you manipulate a stool, the more likely you will lose some organisms (this applies to all parasites in stool). So you may want to eliminate the wash steps and work with the first sediment you obtain from the first spin.

Remember to make the smears thin (this will help you see the spores), and also remember not to decolorize too much. Filtration is not a problem using any of the commercial concentration systems. If you are using gauze, make sure you use woven gauze and use only two layers (do not use pressed gauze, which is too dense and will trap parasites).

8. Comment on the use of formalin within the microbiology laboratory (6–9; this volume)

The formalin regulations were originally developed for industry (plywood, etc.), where large amounts of formalin are used in manufacturing processes. The amount of formalin we are exposed to in the laboratory is minimal; we've never heard of any microbiology laboratory (including a full-service parasitology service) even coming close to the limits.

> The Occupational Safety and Health Administration (OSHA) amended the original regulations for occupational exposure to formaldehyde in May of 1992 [1:Fed Regist 1992 May 27;57(102):22290-328]. The final amendments lower the permissible exposure level for formaldehyde from 1 ppm (part per million) as an 8-hour time-weighted average (TWA) to an 8-hour time-weighted average of 0.75 ppm. The amendments also add medical removal protection provisions to supplement the existing medical surveillance requirements for those employees suffering significant eye, nose or throat irritation and for those suffering from dermal irritation or sensitization from occupational exposure to formaldehyde. In addition, changes have been made to the standard's hazard communication and employee training requirements. These amendments establish specific hazard labeling for all forms of formaldehyde, including mixtures and solutions composed of 0.1% or greater of formaldehyde in excess of 0.1 ppm. Additional hazard labeling, including a warning that formaldehyde presents a potential cancer hazard, is required where formaldehyde levels, under reasonably foreseeable conditions of use, may potentially exceed 0.5 ppm. The final amendments also provide for annual training of all employees exposed to formaldehyde at levels of 0.1 ppm or higher. (6)

Those laboratories that have been monitored have not come close to either measurement. Once a laboratory has been measured and the results (below thresholds for regulatory requirements) are on file, this information does not need to be generated again. No badges are required. Even without a fume hood (many labs do not use a fume hood), performing the routine formalin–ethyl acetate concentration does not seem to be a problem. A number of people who have indicated they want to remove formalin from the laboratory probably don't really understand the history of the regulation or the actual issues. The only possible problem seen in the clinical laboratory/pathology setting might be a routine anatomical pathology laboratory where very large amounts of formalin are used—and with sloppy use. However, even within large microbiology laboratories, it does not seem to be a problem. Appendix B to CFR 1910.1048 (9) contains "Sampling Strategy and Analytical Methods for Formaldehyde." Ultimately, the removal of formalin from the laboratory is based on an individual laboratory decision (keeping in mind the exposure limits indicated below).

> **TWA:** The employer shall ensure that no employee is exposed to an airborne concentration of formaldehyde which exceeds 0.75 parts formaldehyde per million parts of air (0.75 ppm) as an 8-hour TWA (time weighted average).
>
> **STEL:** The employer shall ensure that no employee is exposed to an airborne concentration of formaldehyde which exceeds two parts formaldehyde per million parts of air (2 ppm) as a 15-minute STEL (short-term exposure limit).

Permanent stains

1. What is the purpose of the permanent stained smear?

The purpose of the permanent stained smear (trichrome, iron-hematoxylin) is to provide contrasting colors for both the background debris and parasites present; it is designed to allow examination and recognition of detailed organism morphology under oil immersion examination (100× oil immersion objective for a total magnification of ×1,000). This examination is primarily designed to allow recovery and identification of the intestinal protozoa.

2. How long should one examine the permanent stained smear?

Rather than responding with a specific number of minutes, the recommendation is to examine at least 300 oil immersion (×1,000 total magnification) fields; additional fields may be required if suspect organisms have been seen in the wet preparations from the concentrated specimen.

3. What recent changes have influenced the overall quality of the permanent stained smear?

The use of mercury substitutes in fecal fixatives generally leads to diminished overall morphology quality of the intestinal protozoa. However, some of the mercury substitutes (zinc-based) provide morphologic quality that is close to mercury and allows identification of most of the intestinal protozoa. Differences in detection and identification are usually comparable unless there are very few organisms present or the organisms are quite small. In these circumstances, some organisms may be missed using mercury substitutes. **The development of TOTAL-FIX (no mercury, no formalin, no PVA) provides a fixative that results in excellent overall protozoan morphology.** Although this product is patented (Medical Chemical Corporation, Torrance, CA), there are other fixation options under development with similar characteristics.

4. What is the purpose of the iodine dish in Wheatley's trichrome stain protocol?

Mercury is removed from the smear when placed in the iodine dish; there is a chemical substitution of iodine for mercury. The iodine is removed during the next two alcohol rinses. Therefore, when the slide is placed in trichrome stain, no mercury or iodine remains on the fecal smear.

5. Why don't you need to use the iodine dish when staining fecal smears prepared from specimens preserved in the newer single-vial systems (zinc sulfate-based fixative)?

The zinc sulfate-based fixative is water soluble, so the dry smears can be placed directly into the trichrome dish without having to go through the iodine and subsequent rinse steps. The zinc sulfate will be removed by the water in the trichrome stain.

6. Why might you have to use the iodine dish and subsequent rinses in your staining setup when staining slides from the proficiency testing agencies (AAB, various states, etc.)?

Some smears used for proficiency testing are prepared from fecal specimens that have been preserved in mercury-based fixatives, so the iodine dish and subsequent rinse steps are required to remove mercury and iodine prior to staining with either trichrome or iron hematoxylin stains. For several years, some of the proficiency testing agencies have been using specimens that have been preserved in nonmercury fixatives; thus, the iodine dish is not required.

7. What role does the acetic acid play in the trichrome stain?

Both the trichrome and iron hematoxylin stains are considered regressive stains; the fecal smears are overstained and then destained. The 1% acetic acid in the 90% alcohol rinse step in the trichrome stain removes some of the stain and provides better contrast. However, in some cases differences in the quality of staining between stained protozoa that have been subjected to the 90% alcohol rinse with and without the acetic acid may be difficult to detect. It is important not to destain the smears too much; overall organism morphology will be diminished.

8. What causes the xylene (or xylene substitute) dehydration solutions to turn cloudy when a slide from the previous alcohol dish is moved forward into the xylene dish?

If there is too much water carryover from the last alcohol dish, the xylene solution may turn cloudy. When this occurs, replace the 100% alcohol dishes, back up the slide into 70% alcohol (you can also use a series of steps: 95% then 70%), allow it to stand for 15 min, and then move the slide forward through the 100% alcohol steps and xylene steps.

9. Why is absolute ethanol (100%) recommended as the best approach?

Although many laboratories use the commercially available 95/5% denatured alcohol mix as their "absolute alcohol," the dehydration of stained fecal smears will not be as good as that obtained with 100% ethanol. You may want to add an additional dish of the denatured alcohol (absolute alcohol) to your staining set-up to obtain better dehydration.

10. What is the difference between xylene and xylene substitutes?

There are several differences. As a safety measure, most laboratories have decided to eliminate xylene from their laboratories. However, xylene substitutes generally do not dehydrate as well. Also, after the slides are removed from the last dish of xylene substitute, they take longer to dry. You may want to add an additional dish of the xylene substitute to your staining set-up to obtain better overall dehydration.

11. How do stained fecal smears differ when the fixative does and does not contain PVA?

Although PVA is inert, the material does stain; there will be some background "haze" associated with PVA on the permanent stain. When staining TOTAL-FIX (no PVA present), the background will be quite clean, and the organisms tend to stain a bit more pale. It is important to remember not to destain too long (two quick dips in the 90% alcohol containing 1% acetic acid is sufficient), then immediately move into the first absolute alcohol. Use this dish as a wash, and then begin dehydration in the second absolute alcohol dish; then move smears into the xylene substitute.

STOOL IMMUNOASSAY OPTIONS

1. What are some of the immunoassay options available for stool protozoa? How do they rank in terms of sensitivity and specificity?

Currently, immunoassays are available for *G. lamblia*, *Cryptosporidium* spp., the *E. histolytica*/*E. dispar* group, and *E. histolytica*. Reagents for the detection of *D. fragilis*, *Blastocystis* spp., and the microsporidia are under development. All of the fecal immunoassay test formats are comparable in terms of sensitivity and specificity. Selection of a particular method depends on work flow and the number of test requests and personal preference of that particular laboratory. Outside of the United States (non-FDA approved), there are also reagents available for some of the microsporidia.

2. What methods are available commercially?

Direct fluorescence (DFA), enzyme immunoassay (EIA), and cartridge formats (membrane flow or a solid phase qualitative immunochromatographic procedure) are currently available.

3. Why might someone want to use a fecal immunoassay option?

If the most common organisms found in the area are *Giardia*, *Cryptosporidium*, and/or the *E. histolytica*/*E. dispar* group, then fecal immunoassays are certainly options. Specific patients/history/symptoms would suggest the use of fecal immunoassays. **Both the O&P examinations and fecal immunoassays are recommended for a laboratory test menu; both would be orderable, billable procedures, depending on the patient history and symptoms.**

4. How would stool immunoassay requests fit into a laboratory that also performs O&P examinations?

Any diagnostic laboratory performing routine parasitology testing should offer both options: routine O&P examinations and the fecal immunoassays. **Both the O&P examinations and fecal immunoassays are recommended for a laboratory test menu; both would be orderable, billable procedures.**

5. What do you mean by the "routine O&P examination"?

This test includes (fresh material: liquid or semiliquid stools) a direct wet exam, the concentration, and the permanent stain; preserved specimens would require a concentration and permanent stained smear (no direct wet mount is required).

6. How would you fit the fecal immunoassays into your laboratory?

Per separate requests, you could offer the routine O&P examination, and you could also offer on request the stool immunoassay option. Both options should be in the laboratory ordering test menu.

7. What type of educational initiatives would have to be undertaken prior to offering these options?

Physicians would need to know the pros and cons of ordering either the immunoassay or the routine O&P examination. **Refer to Table A9.1 on test ordering.**

8. What are some of the pros and cons for the fecal immunoassays?

Pros:

- Depending on the format selected, the immunoassays are fast and relatively simple to perform.
- The result can "rule in or out" very specific organisms.
- If the patient becomes asymptomatic at the time the immunoassay is negative, additional testing may not be necessary.
- May help reduce personnel costs (time to perform procedures).
- The fecal immunoassays are more sensitive than the routine O&P exams and/or the special stains for the coccidia or microsporidia.

Cons:

- The fecal immunoassay kits test **only** for selected organisms.
- Depending on the format, they might be somewhat complex to perform.
- Test requests may not justify certain formats (cost, equipment, or training).
- It is critical that the physician realize that a negative immunoassay will not rule out all possible parasitic etiologic agents causing diarrhea.
- ***Giardia lamblia* may require a fecal immunoassay on two different stool specimens to get a positive result. This is not the case for other protozoa** (10, 11).

9. Can the fecal immunoassays be used for duodenal fluid (giardiasis)?

First, the tests have NOT been approved or validated for this type of specimen. Also, the duodenal fluid/aspirate would contain primarily the trophozoite form, not the cyst stage for which the reagents have been designed. An example: if one is using the FA (rather than EIA or cartridge), the trophs may appear to be fluorescent at a very pale 1+, while the cysts are a strong 3 to 4+. So while there may be a few antigenic sites that are shared by the cysts and trophs, the commercial tests for *Giardia* are designed primarily to detect the cyst antigens.

You can try the reagents on these specimens, but if the result is negative, the result in no way has ruled giardiasis out. I would recommend testing stool only; if the result is positive, this ap-

TABLE A9.1 Stool testing order recommendations (for physicians)

Patient and/or situation	Test ordered[a]	Follow-up test ordered
Patient with diarrhea and AIDS or other cause of immune deficiency Potential waterborne outbreak (municipal/city water supply)	*Cryptosporidium* or *Giardia*/*Cryptosporidium* immunoassay	If immunoassays are negative and symptoms continue, special tests for microsporidia (modified trichrome stain) and other coccidia (modified acid-fast stain) and O&P exam should be performed
Patient with diarrhea (nursery school, day care center, camper, backpacker) Patient with diarrhea and potential waterborne outbreak (resort setting) Patient with diarrhea from areas where *Giardia* is the most common parasite found	*Giardia* or *Giardia*/*Cryptosporidium* immunoassay (perform testing on two stools before reporting as negative) **Particularly relevant for areas of the United States where *Giardia* is the most common organism found**	If immunoassays are negative and symptoms continue, special tests for microsporidia and other coccidia (see above) and O&P exam should be performed
Patient with diarrhea and relevant travel history Patient with diarrhea who is a past or present resident of a developing country Patient in an area of the United States where parasites **other than** *Giardia* are found	O&P exam, *Entamoeba histolytica*/*E. dispar* immunoassay; immunoassay for confirmation of *E. histolytica*; various tests for *Strongyloides* may be relevant (even in the absence of eosinophilia)	If exams are negative and symptoms continue, special tests for coccidia and microsporidia should be performed
Patient with unexplained eosinophilia and possible diarrhea; if chronic, patient may also have history of respiratory problems (larval migration) and/or sepsis or meningitis (hyperinfection)	Although the O&P exam is a possibility, the agar plate culture for *Strongyloides stercoralis* (more sensitive than the O&P exam) is recommended	If tests are negative and symptoms continue, additional O&P exams and special tests for microsporidia and other coccidia should be performed
Patient with diarrhea (suspected foodborne outbreak)	Test for *Cyclospora cayetanensis* (modified acid-fast stain, autofluorescence)	If tests are negative and symptoms continue, special procedures for microsporidia and other coccidia and O&P exam should be performed

[a] Depending on the particular immunoassay kit used, various single or multiple organisms may be included. Selection of a particular kit depends on many variables: clinical relevance, cost, ease of performance, training, personnel availability, number of test orders, training of physician clients, sensitivity, specificity, equipment, time to result, etc. Very few laboratories will handle this type of testing exactly the same. Many options are clinically relevant and acceptable for good patient care. It is critical that the laboratory report indicate specifically which organisms could be identified using the kit; a negative report should list the organisms relevant to that particular kit.

proach might avoid the need for duodenal aspirate testing altogether.

10. Comment on the use of the *Giardia* fecal immunoassay; how many specimens should be tested before assuming the patient is negative?

Since the evidence indicates that *Giardia* is shed sporadically, and that more than one immunoassay might be required to diagnose the infection, the recommendation is similar to that for stool collection for the routine O&P examination: **for *Giardia*, perform the immunoassay on two different stools (assuming the first specimen is negative),** collected within no more than a 10-day period; a good collection schedule would be day one, then day three or four. That way, one would assume one of those collections would yield a positive result if the organisms (in sufficient numbers) are present. However, you also have to remember that **if the patient is a carrier with a low organism load, even the second immunoassay might be negative. Note: One stool is sufficient for immunoassay testing for *Cryptosporidium* spp.**

11. Although an FA for *Cryptosporidium/Giardia* was negative, the O&P concentrate showed *Giardia* trophs on the wet mount. Might we miss positive *Giardia* if only the FA is ordered? Also, do any of the EIA antigen tests detect both trophs and cysts, and should we switch to one of those?

Although the antibody in the immunoassay kits is to the cyst antigen (primarily, but it is a polyclonal reagent), in some of the kits the trophs do fluoresce, but much less intensely (around a 1+, maybe even a 2+, thus indicating some shared epitopes). In almost all patients, there will be a combination of cysts and trophs, unless the patient has active diarrhea and is passing only trophs (no time for cysts to form with rapid passage through the

GI tract). However, most patients will have cysts as well, and the results (if above the test limits for sensitivity) will be positive. The situation you describe can happen, but it's probably not that common. **However, with ONLY trophs present, you may get a negative result. Some of the kits tend to provide a bit higher fluorescence with the troph, but it varies.**

12. After the patient has been treated, how long will the *Giardia* antigen test remain positive?

It has been recommended that you test about 7 days after therapy, hopefully to avoid picking up residual antigen. However, if you wait too long (several weeks), you run the risk of picking up antigen from a possible reinfection. Some people believe that low antigen levels can be found for up to about 2 weeks. A good time frame for retesting would probably be about 7 to 10 days after therapy. If the first specimen at 7 days is still positive, then you could retest at 10 to 12 days. **Also, we know that the immunoassays (for diagnosis) may not pick up low antigen loads (organism shedding issues), and thus the recommendation for diagnostic immunoassay testing for giardiasis has been changed, and performing immunoassay testing on one additional stool specimen (if the first one is negative) is recommended. The testing on two different stool specimens should be performed within about 3 to 5 days.**

13. Why do fecal immunoassay kits that test for either *Entamoeba histolytica* or the *Entamoeba histolytica/E. dispar* group require fresh or frozen stools or stools submitted in Cary-Blair?

Unfortunately, at the present time, these reagents will not function properly on preserved fecal specimens. Although the manufacturers are trying to develop such kits, they are not yet available.

14. How long will antigen survive in fresh stools?

It is recommended that fresh stools be tested within 24 h of collection; they can be stored overnight in the refrigerator. They can also be frozen or preserved in 10% formalin prior to testing (both freezing and/or formalin preservation methods will preserve antigen for long periods of time—even years).

ORGANISM IDENTIFICATION (1–4; this volume)

Protozoa

1. What is the most effective technique for the identification of the intestinal protozoa?

Although some protozoan cysts can be seen and identified on the wet preparation smear (direct mount, concentration sediment wet mount), the permanent stained smear is the procedure that is recommended as the most relevant and accurate for the identification of the intestinal protozoa. These preparations are examined using the oil immersion objective (100×) for a total magnification of ×1,000. At least 300 oil immersion fields should be examined prior to reporting the permanent stained smear result.

2. Are trophozoites ever seen in the wet mounts of stool?

Usually the trophozoites are not seen in the concentration sediment wet mount preparations unless they are prepared from stool preserved in a fixative containing no formalin. Motile trophozoites can occasionally be seen in the direct wet smear prepared from fresh soft or liquid stool that is more likely to contain trophozoites, but this is rare.

3. What are some tips to consider when reporting *Entamoeba hartmanni*?

The measurements of 10 µm or less for the *E. hartmanni* cyst refer to wet preparation measurements, so the measurement should be decreased ~1 µm on the permanent stained smear. When you see a cyst on the permanent stained smear, there is often a halo representing shrinkage. **The cyst needs to be measured to include that halo.** On the bench, the measurements for this cyst generally run from around 9.5 down to about 8 µm, and they are morphologically definitely *E. hartmanni*. The *E. hartmanni* cyst generally contains more chromatoidal bars than are seen in *E. histolytica/E. dispar* and often stops at two nuclei, rather than four. Also, the *E. histolytica/E. dispar* cysts tend to measure routinely on the bench (trichrome slides) from about 10.5 up to about 13 µm or so. Also, on the bench, the *E. hartmanni* nucleus in the trophozoite will tend to look like a bull's-eye—very sharp line of nuclear chromatin with the karyosome right in the middle.

4. Do nonpathogenic protozoa ever cause symptoms?

Endolimax nana, *Iodamoeba bütschlii*, *Chilomastix mesnili*, and *Pentatrichomonas hominis* (for example) have been categorized as nonpathogens. Although rare, patients have been documented who have been symptomatic with a nonpathogen. However, it is sometimes difficult to determine from the case history the extent of the workup, including coccidia and the microsporidia. Before assigning symptoms to nonpathogenic protozoa, a comprehensive search for other proven pathogens should be performed.

5. What are some of the considerations when reporting *Entamoeba histolytica* versus the *Entamoeba histolytica/E. dispar* group?

Morphologically, unless the trophozoite measures >12 µm and contains ingested RBCs, the identification must be *Entamoeba histolytica/E. dispar*, indicating that the true pathogen (*E. histolytica*) cannot be differentiated from the nonpathogen (*E. dispar*). One can also report the true pathogen if the fecal immunoassay specific for *E. histolytica* is positive. Since *Entamoeba moshkovskii* and *Entamoeba bangladeshi* also look very much like others in the *Entamoeba* genus, some people have suggested that they should be reported as the "*Entamoeba histolytica* complex."

6. What color is the autofluorescence seen with *Cyclospora cayetanensis*?

The color depends on the particular FA filters used. If Calcofluor white filters are used, the oocysts will appear as pale blue rings; if FITC filters are used, the oocysts will appear to be more yellow-green. Fluorescence intensity will vary from about 1+ to 2+; it is rare to see stronger autofluorescence with these oocysts.

7. Why is *Blastocystis hominis* so controversial regarding pathogenicity?

What we currently call *Blastocystis hominis* (morphologically) appears to be approximately 10 to 20 different strains, subtypes, or species, some of which are pathogenic and some of which are nonpathogenic. Based on continuing molecular studies, changes in the classification of this group are probable. If all strains, subtypes, and species currently make up what is called "*Blastocystis hominis*," this could explain the controversy regarding pathogenicity and the fact that some patients are symptomatic, while some are asymptomatic. Unfortunately, all of the strains, subtypes, and species are morphologically identical; therefore, the correct identification reported continues to be *Blastocystis* spp. (*B. hominis*), and the number should be quantitated from the permanent stained smear (rare, few, moderate, many, packed). Many laboratories have begun reporting *Blastocystis* spp., with a report comment explaining the pathogenic versus nonpathogenic controversy. This is the recommended approach.

Helminths

1. Why can't all helminth eggs be recovered using the flotation concentration rather than the sedimentation concentration?

Some helminth eggs are quite heavy (e.g., unfertilized *Ascaris* eggs) and will not float, even using zinc sulfate with a specific gravity of 1.20. Other helminth eggs are operculated; when the egg is placed in a high specific gravity solution, the operculum "pops" open and the egg fills with fluid and sinks to the bottom of the tube. Thus, both the surface film and the sediment should be examined before reporting the specimen as negative.

2. Why do helminth larvae need to be identified to the species level; shouldn't all larvae recovered in stool be that of *Strongyloides stercoralis*?

Although helminth larvae in stool are normally *Strongyloides stercoralis*, there is always the possibility that in fresh stool hookworm eggs have continued to mature and may hatch before the stool is processed and/or placed in fixatives. It is important to make sure that the larvae seen are, in fact, the rhabditiform (noninfectious) larvae of *S. stercoralis* rather than larvae of hookworm. The agar plate culture is the most sensitive method for the recovery of *S. stercoralis* larvae. Also, remember that migrating nematode larvae could also be recovered from respiratory specimens (sputum, BAL, etc.).

3. Are there any specific recommendations for the detection of schistosome eggs?

When trying to diagnose schistosomiasis, regardless of the species suspected, you should examine both stool (several different stool specimens) and urine (spot urines plus 24-hour specimen) (collected with no preservatives). Occasionally, adult worms get into blood vessels, where they aren't normally found (example: *Schistosoma mansoni* eggs found in urine only). When you perform a sedimentation concentration, use saline to prevent premature egg hatching. Once you are ready to try a hatching procedure, then you can put the sediment into spring water (dechlorinated water) to stimulate hatching. When examining wet mounts under the microscope, you need to look for the movement of cilia on the miracidium larvae within the egg shell (thus the need to collect the specimens with no preservatives). You want to be able to tell the physician whether the eggs, if present, are viable or all you see are dead egg shells. If you suspect schistosomiasis, it is recommended that you examine a number of wet mounts, particularly if you aren't going to perform a hatching test.

4. What is the most sensitive test for the diagnosis of strongyloidiasis?

Agar plate cultures are recommended for the recovery of *Strongyloides stercoralis* larvae and tend to be more sensitive than some of the other diagnostic methods such as the O&P examination. Stool is placed onto agar plates, and the plates are sealed to prevent accidental infections and held for 2 days at room temperature. As the larvae crawl over the agar, they carry bacteria with them, thus creating visible tracks over the agar. The plates are examined under the microscope for confirmation of larvae, the surface of the agar is washed with 10% formalin, and final confirmation of larval identification is made via wet examination of the sediment from the formalin washings.

In a study looking at the prevalence of *S. stercoralis* in three areas of Brazil, the diagnostic efficacy of the agar plate culture method was as high as 93.9% compared to only 28.5% and 26.5% by the Harada-Mori filter paper culture and fecal concentration methods, respectively, when fecal specimens were processed using all three methods. Among the 49 positive samples, about 60% were confirmed as positive using only the agar plate method. These results indicate that the agar plate approach is probably a much more sensitive diagnostic method and is recommended for the diagnosis of strongyloidiasis.

It is important to remember that more than half of *S. stercoralis*–infected individuals have low-level infections. The agar plate method continues to be documented as a more sensitive method than the usual direct smear or fecal concentration methods. Daily search for furrows on agar plates for up to 6 consecutive days results in increased sensitivity for diagnosis of both *S. stercoralis* and hookworm infections. **Also, a careful search for *S. stercoralis* should be made in all patients with comparable clinical findings before deciding on a diagnosis of idiopathic eosinophilic colitis, because consequent steroid treatment may have a fatal outcome by inducing widespread dissemination of the parasite.** Serologic testing for antibody is also recommended if strongyloidiasis is still suspected in the absence of laboratory confirmation of larvae.

5. Where can one get serologies for a *Baylisascaris procyonis* infection?

CDC:
1-404-718-4745 (M-F 7:30 am – 4 pm EST)
After-hours emergencies: 1-770-488-7100

REPORTING

Organism

1. Should common or scientific names be used when reporting the presence of parasites?

The scientific name (genus, species) should be used on the final report that goes to the physician and to the patient's chart. It is also recommended that the stage of the organism be included (trophozoite, cyst, oocyst, spore, egg, larvae, adult worm); various stages of the malaria parasites will impact therapy (see section on blood parasites in this appendix, below).

2. What happens if there are several different names used in the literature (*Giardia lamblia, G. intestinalis, G. duodenalis*)?

It is appropriate to use the most commonly accepted name (*G. lamblia*); you can also let the proficiency testing organism lists be your guide. When a replacement name begins to appear on the proficiency test list, then it's appropriate to notify your clients and institute the name change. Remember, it is very important to notify all clients prior to making any name changes.

3. How has the reporting of *Cryptosporidium parvum* changed, and why?

More than one species of *Cryptosporidium* can cause disease in humans (*C. parvum*: humans and other mammals; *C. hominis*: humans; and other less common species). However, the different species cannot be differentiated on the basis of morphology. **It is now recommended that the more correct reporting format would be *Cryptosporidium* spp. (rather than *C. parvum*).**

4. Should WBCs and/or other cells or yeast be reported, and why?

The reporting and quantitation (rare, few, moderate, many) of WBCs (polymorphonuclear leukocytes [PMNs], macrophages, eosinophils) provide some additional information for the physician. If the patient continues to have diarrhea, it may give the physician something more to consider, particularly if stool culture has not been ordered. Also, conditions related to noninfectious diarrheas may also result in WBCs and macrophages in the stool. We always include this type of information/explanation in our educational process regarding reporting formats. Physicians can then decide on the relevance of the information. Remember that when reporting WBCs we can also identify eosinophils from the permanent stained smear (may or may not be related to parasitic infection and may or may not correlate with peripheral eosinophilia; this information may also be helpful).

Reporting yeast is a bit different. To report anything about yeast, you must know the stool was fresh or immediately put in preservative. If there are lots of yeasts, budding yeasts, and/or pseudohyphae, then this provides some additional information for the physician, often depending on the patient's general condition and whether or not the patient is immunosuppressed. However, if you don't know whether the collection criteria were met, then reporting anything about yeast is NOT recommended, since this type of report may be misleading. Certainly, in these circumstances, the physician can be notified directly.

5. How should *Blastocystis hominis* be reported?

In the past there was some agreement that the number of organisms present (mod, many, packed) was more likely to be associated with symptoms. However, in the past few years, there have been anecdotal reports of patients being symptomatic with rare or few organisms. The current recommendation is to report the organism and quantitate per rare, few, mod, many, packed (using your proficiency testing quantitation scheme). It is important to confirm that the physicians know what the report means and understand the pathogenicity issues surrounding this organism; they can then correlate the numbers with symptoms. If rare or few, there are no solid data per number relevance other than the anecdotal case reports (many of which are word of mouth). Many physicians treat if the patient is symptomatic and no other organisms are found (including coccidia and/or microsporidia).

"*Blastocystis hominis*" includes several strains, subtypes, and species, some of which are pathogenic and some of which are nonpathogenic. Based on continuing molecular studies, changes in the classification of this group could be updated. If pathogenic and nonpathogenic strains, subtypes, or species make up what is morphologically called "*Blastocystis hominis*," this could explain the controversy regarding pathogenicity and the fact that some patients are symptomatic, while some are asymptomatic. Unfortunately, strain, subtype, and species differences cannot be detected by morphology; they look alike. **You should begin reporting *Blastocystis* spp. rather than *B. hominis*. It is also recommended that a report comment be included regarding strains, subtypes, or species and pathogenicity.**

6. How should intestinal protozoa be reported?

Reporting trophozoites/cysts: This has been the accepted way of reporting intestinal protozoa for a couple of reasons. First, different drugs are used to treat *Entamoeba histolytica* cysts versus trophs. Also, since the cyst form is the infective stage for the protozoa (exception: the trichomonads), the report does convey some epidemiologic information. For these reasons, the current recommendation is to continue to report all protozoa (genus, species, and stage).

7. What additional reporting comments should be added to the reporting of the O&P examination and the fecal immunoassays?

It is important to add the following comment to the O&P examination:

> "The O&P examination is not designed to detect the intestinal coccidia (*Cryptosporidium* spp., *Cyclospora cayetanensis*) or the microsporidia. *Cystoisospora belli* oocysts (coccidia) can be detected from the concentration sediment examination."

Report the results of the fecal immunoassays based on the specific organism(s) relevant to the kit:

> "No *Giardia lamblia* antigen detected." OR "*Giardia lamblia* antigen present."
>
> "No *Giardia lamblia* or *Cryptosporidium* spp. antigen detected."

OR

> "Negative for *Giardia lamblia*."
>
> "Negative for *Giardia lamblia* and *Cryptosporidium* spp."

The report of* Blastocystis *spp. should be accompanied by the following report comment:

> "*Blastocystis* spp. (morphologically) comprise a number of different strains, subtypes, or species, some of which are pathogenic and some of which are nonpathogenic. This explains why some patients are symptomatic and some are asymptomatic."

Quantitation

1. What organisms should be quantitated in the final report to the physician and patient's chart?

Organisms and nonorganism cells/structures (stool blood cells, Charcot-Leyden crystals) that are recommended for quantitation include the following:

A. Intestinal protozoa: *Blastocystis* spp.
B. Helminths: *Trichuris trichiura* eggs, *Clonorchis sinensis* eggs, schistosome eggs (also report viability of *Schistosoma* spp. eggs)
C. Blood parasites: all malaria organisms and *Babesia* spp.
D. Blood cells (PMNs, macrophages, RBCs)
E. Charcot-Leyden (CL) crystals

2. Why are all proficiency testing (PT) specimen answers reported and quantitated, while clinical specimen reports are rarely quantitated in terms of organism numbers?

There are two issues to consider.

1. When reporting PT specimen results, you are asked to quantitate organisms in both the formalin wet mounts and permanent stained smears. This information serves as a **quality control check for the proficiency testing agency to ensure that organism numbers are consistent throughout the challenge vials/slides.**
2. Most laboratories (with few organism exceptions) do not quantitate organisms on the concentration or permanent stained smear reports. Some exceptions might be *Trichuris trichiura* eggs in a concentration wet mount (light infections might not be treated). Since many organisms are shed on a random basis, quantitation may change dramatically from day to day and generally has little clinical relevance.

PROFICIENCY TESTING (1–5, 12; this volume)

Wet Preparations

1. How should you examine a wet preparation for proficiency testing?

Most directions recommend that you shake the vial and take a sample from the mixed vial contents. However, if the number of eggs is few, you may be better off taking a very small drop from the settled material. If you take too large a drop, it will be too thick to examine properly. Make sure you do not add too much iodine; very darkly stained helminth eggs can resemble debris.

2. Are there any tips regarding the microscope setup for the examination of an unstained wet preparation?

The microscope should be aligned properly using Köhler illumination. Make sure the light is not too bright; otherwise, you may shine right through the organisms and miss the parasites. Also, you may want to close the diaphragm a bit to provide more contrast. Extra contrast is particularly important when reading with saline only (no iodine added).

PERMANENT STAINED SMEARS

1. How should you examine a permanent stained fecal smear for proficiency testing?

Make sure the light is very bright (condenser all the way up) and the diaphragm is open. Review both thin and thick areas of the smear; examine at least 300 oil immersion fields (using the 100× oil immersion objective) before you report the specimen as a negative. If you use the 50× or 60× scanning oil immersion lens, you still need to review 300 oil fields as indicated above (using the 100× oil immersion objective).

TISSUES OR FLUIDS (1–4, 12; this volume)

1. How should tissues be submitted to the laboratory?

Tissue specimens should be immediately sent to the laboratory and kept moist during transit. If the tissue will be processed for culture (*Acanthamoeba, Naegleria, Leishmania* spp., *Trypanosoma* spp., or *Toxoplasma gondii*), the specimen should be kept sterile and submitted in a sterile container. If the tissue will be processed for wet examinations and permanent stained smears, any type of container is acceptable. Remember that the specimen must be kept moist; if the specimen dries out during transit, neither culture nor stained smears will provide acceptable results.

2. How should eye specimens be submitted for *Acanthamoeba* culture?

If sending the specimen in a tube/vial or screw-cap tube, make sure the container is filled with transport fluid (saline is acceptable). This will prevent the small tissue specimen from drying out if during transit it is shaken onto the side of the container. If the container is not full of fluid, the tissue may dry out and will be unacceptable for culture.

3. How should duodenal drainage specimens be handled?

Duodenal drainage specimens must be submitted to the laboratory as quickly as possible for processing. Delays may prevent organism recovery and identification. These specimens may be very liquid and may contain a lot of mucus. It is best to centrifuge the tube, discard any supernatant fluid left, and examine the mucus only as wet preparations (low light) and/or permanent stained smears (trichrome, iron hematoxylin). Do not use ethyl acetate in the concentration procedure; a single spin is recommended (do not “rinse” the specimen).

BLOOD PARASITES (1, 2, 13–16; this volume)

Many questions have been asked regarding various aspects of diagnostic medical parasitology and the examination of blood specimens. Answers to these questions may be helpful for those working in this area of microbiology and may provide some “tips of the trade” that are learned through many years of bench experience.

SPECIMEN COLLECTION

Blood

1. If *Plasmodium falciparum* parasites are sequestered in the capillaries, why not do a finger stick rather than a venipuncture?

The capillaries are generally in the deep tissues (spleen, liver, bone marrow), so a finger stick blood sample is no more likely to be positive than venipuncture blood. There may be some minor differences with *P. falciparum*, but not sufficient to warrant eliminating venipunctures. The anticoagulant tube also provides additional blood for multiple thick and thin blood films and/or buffy coat films.

2. What is the best anticoagulant to use for blood specimens?

Although heparin (green top) or EDTA (lavender top) can be used, EDTA is recommended as providing better organism morphology, particularly for *Plasmodium* spp. Blood collected using EDTA anticoagulant is acceptable; however, if the blood remains in the tube for >1 to 2 hours, true stippling may not be visible within the infected RBCs (*Plasmodium vivax*, *Plasmodium ovale*), organism distortion occurs, and **parasite numbers actually decrease after 4 to 6 hours.** Also, when using anticoagulants, the proper ratio between blood and anticoagulant is necessary for good organism morphology. The lavender-top tube should be filled with blood to provide the proper blood-anticoagulant ratio. Heparin can also be used, but EDTA is preferred. Finger-stick blood is recommended when the volume of blood required is minimal (no other hematologic procedures have been ordered). The blood should be free-flowing when taken for smear preparation and should not be contaminated with alcohol used to clean the finger prior to the stick. However, finger stick blood is no longer commonly used in many parts of the world. When blood is collected in EDTA, specimens should be processed immediately after blood collection. **Parasite numbers may decrease if processing is delayed, even 4 to 6 hours.** Adhesion of the blood to the slide can be a problem if the ratio of anticoagulant to blood is high, the patient is anemic, or the blood was held in EDTA too long. See Table A9.2.

3. Why do new slides have to be precleaned with alcohol prior to use?

Even new slides will be coated with a very fine layer of oil (to allow the slides to be "pulled apart" one from the other); removing the greasy coating will cause the blood to flow more smoothly over the glass during blood film preparation. "Holes" in the blood film are evidence of oil or grease on the slide. **Use Standard Precautions:** remember, both slides and spreaders must be held by the edges and not touched on any part of the slide that will come in contact with blood.

4. When should blood specimens be drawn for a suspect malaria diagnosis?

The majority of patients we see in the United States with malaria have never been exposed to the organism before, so they have no antibody. When they are seen in the ER or clinic **they do NOT have a synchronized fever cycle.** These **immunologically naive** patients may present with nonspecific symptoms that can mimic many other diseases. The rule of thumb is to **draw immediately;** do not wait for some "magic" periodic cycle that may never appear. Patients with a very low parasitemia with *P. falciparum* can become quite ill before they have any type of fever cycle or gametocytes. With any patient when malaria is suspect or the patient has an FUO (fever of unknown origin), blood should be drawn and both thick and thin blood films prepared and examined immediately. **This request is always considered a STAT request.** In areas of endemicity where people have been exposed to the parasite before (have some antibody), they may not become symptomatic until they actually have some sort of periodic fever cycle. However, you should always use the general guideline to

TABLE A9.2 *Plasmodium* morphology in EDTA

Organism	Morphology (normal)	Morphology (EDTA) (contact with EDTA for >1–2 hours)
P. falciparum		
Rings	Typical, small to medium rings; double rings/cell	Numbers tend to remain constant; all parasites continue to grow in EDTA
Gametocytes	Crescent-shaped	Gametocytes may round up and be confused with other species
P. vivax		
Trophozoites	Ameboid trophozoites, for both early and late rings	May round up and lose their characteristic shape
Schüffner's dots	Typical dots appear in late rings; present throughout the rest of the life cycle stages	Stippling (Schüffner's dots) may not be visible at all, including all life cycle stages (regardless of buffer pH used) **NOTE**: If slides are prepared soon after collection in EDTA, the dots will be seen after staining; however, if slides are prepared after the blood has been in contact with EDTA for several hours, the dots may not be visible after staining
Plasmodium spp.		
Male gametocytes	No exflagellation	As blood cools and becomes oxygenated, the parasites "think" they are now in the mosquito (this cycle may continue). The male gametocyte may exflagellate; microgametes may resemble *Borrelia* (also related to pH and pCO_2—lid left off tube).

draw immediately. Also, remember that one set of negative blood films does not rule out malaria. If the first set (both thick and thin films) is negative, you can recommend that additional blood films be drawn in about 4 to 6 hours. Also, any decision to delay treatment should be left to the physician, not the laboratory.

SPECIMEN PROCESSING

1. Why is it important that the EDTA blood be processed as quickly as possible?

If a tube of blood containing EDTA cools to room temperature and the cap has been removed, several parasite changes can occur. Due to temperature and CO_2 changes, the parasites within the RBCs will respond as if they were now in the mosquito after being taken in with a blood meal. The morphology of these changes in the life cycle and within the RBCs can cause confusion when examining blood films prepared from this blood.

A. Stippling (Schüffner's dots) may not be visible.
B. The male gametocyte (if present) may exflagellate.
C. The ookinetes of *Plasmodium* species other than *P. falciparum* may develop as if they were in the mosquito and may mimic the crescent-shaped gametocytes of *P. falciparum*.
D. Smears left longer than 24 hours can autofix; if this occurs, lysis of the RBCs will be difficult, if not impossible, to achieve. This tends to occur more quickly in warm, humid climates.
E. Thick smears can be dried in a 37°C incubator for 10 to 15 min without fixation of the RBCs; **do not go beyond 15 min using this method.**

2. Why is it important to keep thick films from getting hot (heat fixation)?

Heat will fix the RBCs, and they subsequently will not lyse in the staining process. (See item E above.)

3. What should you do if you have blood films that can't be stained for several days?

If staining with Giemsa will be delayed for more than 3 days or if the film will be stained with Wright's stain, lyse the RBCs in the thick film by placing the slide in buffered water (pH 7.0 to 7.2) for 10 min, remove it from the water, and place it in a vertical position to air dry. The laked thick films and thin films should be dipped in absolute methanol and placed in a vertical position to air dry. This is particularly important if the blood films will be stored for days or weeks prior to staining. **Processing of patient specimens for diagnosis needs to be performed on a STAT basis; it is inappropriate to hold slides for staining unless they are for teaching purposes and time is not a critical issue. However, the longer the slides are held, the more likely there will be a decrease in overall staining quality.**

DIAGNOSTIC METHODS

1. Why is it important to always examine both thick and thin blood films prior to reporting the specimen as negative for blood parasites?

Thick blood films allow a larger amount of blood to be examined, which increases the possibility of detecting light infections. However, only experienced workers can usually make identifications to the species level from a thick film, particularly for malaria parasites. The morphologic characteristics of blood parasites and identification to the species level are best accomplished from the thin films. However, it is mandatory that both types of films be examined prior to reporting negative findings.

2. What are the advantages of the thick blood film?

The aim of the preparation of a thick film is to have a drop of blood with 20 or 30 layers of RBCs on the slide, then to lyse the red cells, wash off the hemoglobin, and stain the parasites, which remain intact in the process. As a consequence, RBCs will not normally be visible, but WBCs and parasites will be seen. It is essential to get used to the characteristics of malaria parasites and know what to look for on such a preparation. An experienced microscopist should be able to detect 20 parasites per µl of blood (i.e., a parasitemia of 0.0001%), having examined 100 fields in 10 min. A greater volume of blood can be examined in the same amount of time taken for examination of the thin blood film. The presence of phagocytized malaria pigment within WBCs (particularly in cases with low levels of parasitemia) can be very helpful. Occasionally, one can see Schüffner's dots in a thick film. However, recognition of the malarial pigment within WBCs and Schüffner's dots in a thick blood film may be difficult.

3. What are the disadvantages of the thick blood film?

Due to lysis of the RBCs in processing thick blood films, the thick film cannot be used to compare the size of the parasite within the RBC or to compare the size of the infected RBC to the uninfected RBCs. Recognition of organism distortion and identification to the species level are generally more difficult from the thick film.

4. What are some of the problems associated with thick blood films? (17)

The thick film flakes off during the staining process.

a. Film was not dry.
b. Film did not dry evenly.
c. Blood film was too thick (refer to combination thick-thin blood film protocol).
d. The blood was too diluted with anticoagulant or the patient was anemic (centrifuge blood at 500 × *g* for 1 min and repeat the thick film).
e. Slides were greasy or dirty.
f. Blood was in EDTA too long (prepare thick films thinner than normal; dry for a longer period of time).

The thick film does not stain adequately.

g. Blood film was too thick.
h. Staining solution was too dilute or staining time too short.

5. What are some tips for improving thick blood films?

After the thick films are dry, fix the smear with acetone for a few seconds (**only dip twice**) and then air dry the film. This approach will improve the durability of the thick film and will **not** interfere with the lysis of the RBCs. These films tend to have a clean background, making the parasites easier to see. Overall adherence to the slide is enhanced using this approach.

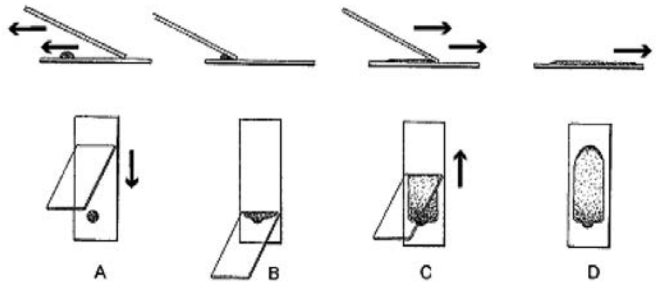


Figure A9.1 The traditional method for preparing a thin blood film. The blood can be either "pushed" or "pulled" by the spreader slide. (Illustration by Sharon Belkin.) Note that "D" illustrates a thin film with a good feather edge. doi:10.1128/9781555819002.Apx9.f1

6. How do you prepare thin blood films?

Keep the spreader at about a 40 to 45° angle to allow blood to flow to **almost the edge of the slide.** Lower the angle to about 30 to 35° to pull the film in one smooth motion (Fig. A9.1). The finished blood film should appear as a feathered edge in the center of the slide, having a **free margin** on either side. Before you fix the thin blood films in absolute methanol, the film must be completely dry. If slides must be stored unfixed, they should be frozen. Fixation too long (>30 to 45 s): stippling may be reduced. Fixation too short: RBCs may be distorted (crescent-shaped) or partially lysed. Fixation: It is better to use a dispensing bottle for methanol, rather than a Coplin jar, in which the methanol will pick up water from the air. Methanol used one day (Coplin jar) should not be reused the next day; begin with fresh stock.

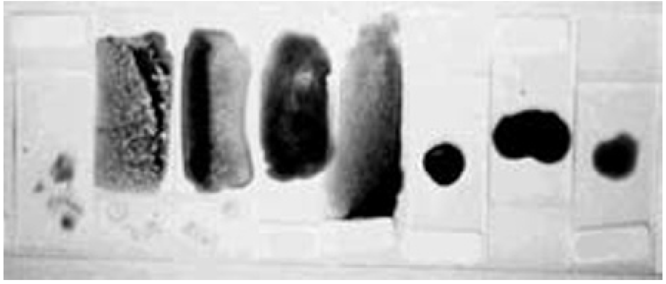
7. What are the advantages of the thin blood film?

The RBC morphology can be seen, as well as the size relationship of the parasite within the infected RBC. The sizes of the infected RBCs can be compared to that of the uninfected RBCs. It is much easier to identify malaria organisms to the species level from the thin blood film. The parasitemia can be much more easily calculated from the thin blood film than the thick film.

8. What are the disadvantages of the thin blood film?

This method has a much lower sensitivity than the thick blood film; thus, infections with a low parasitemia may be missed.

Figure A9.2 Poorly prepared thin and thick blood films (dirty slides, oil on slides, too thick preparations, poor spreading of the blood); organism morphology will be very poor on the stained films. doi:10.1128/9781555819002.Apx9.f2



9. What are some of the problems associated with thin blood films? (Poorly prepared thick and thin blood films can be seen in Fig. A9.2.) (17)

Films are too thick:

a. Too large a drop of blood was used.
b. The RBCs may be crowded together with no thin feathered edge.
c. The film was prepared too quickly or with too large an angle >30 to 35°.

Films are too thin:

d. Blood from anemic patients will not spread well; spread the blood more quickly. Allow the blood to settle or centrifuge, remove some plasma, and repeat the thin film.
e. Angle between the spreader and slide was too small <30°.

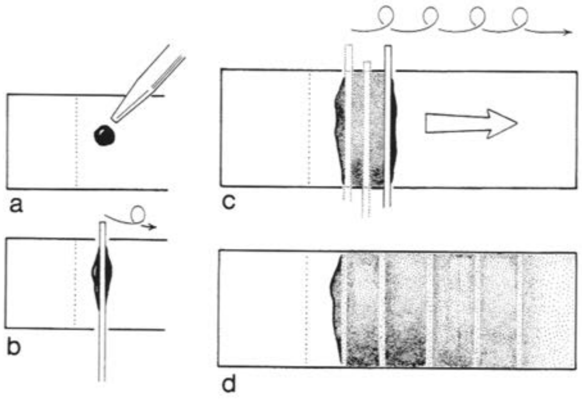
Smears have ragged edges:

f. The drop of blood may have been in front of the spreader.
g. The slides were greasy or dirty.
h. The spreader slide was chipped.
i. The spreader slide was reused (if possible, use a new slide each time).
j. If the blood contained no anticoagulant, a delay in spreading may have led to fibrin formation, causing streaking.
k. May also be due to high plasma fibrinogen level and **not technique.**

10. How does one prepare a combination thick/thin blood film?

The combination thick-thin blood film provides both options on one glass slide, and the slide can be stained as either a thick or thin blood film (Fig. A9.3). If fixed prior to staining, then the

Figure A9.3 Method of thick-thin combination blood film preparation. (a) Position of drop of EDTA-containing blood. (b) Position of the applicator stick in contact with blood and glass slide. (c) Rotation of the applicator stick. (d) Completed thick-thin combination blood film prior to staining. (Illustration by Sharon Belkin.) doi:10.1128/9781555819002.Apx9.f3



smear will be read as a thin blood film; if RBCs are lysed during staining, the preparation will be read as a thick blood film (parasites, platelets, WBCs). This combination blood film dries more rapidly than the traditional thick blood film, thus allowing staining and examination to proceed with very little waiting time for the slide(s) to dry.

A. Place a drop (30 to 40 µl) of blood onto one end of a clean slide about 0.5 in. from the end.

B. Using an applicator stick lying across the glass slide and keeping the applicator in contact with the blood and glass, rotate (do not roll) the stick in a circular motion while moving the stick down the glass slide to the opposite end.

C. The appearance of the blood smear should be alternate thick and thin areas of blood.

D. Immediately place the film over some small print and be sure that the print is just barely readable.

E. Allow the film to air dry horizontally and protected from dust for at least 30 min to 1 hour. Do not attempt to speed the drying process by applying any type of heat, because the heat will fix the RBCs and they subsequently will not lyse in the staining process.

F. This slide can be stained as either a thick or thin blood film.

11. How should malaria smears be stained with Giemsa stain?

Giemsa is a mixture of eosin and methylene blue. Stock solutions of Giemsa may be purchased commercially. Some brands are better than others. The stock solution of Giemsa stain is easily prepared from commercially available Giemsa powder.

Dilutions of Giemsa stain: Stock solutions of Giemsa stain must always be diluted by mixing an appropriate amount of stain with distilled neutral or slightly alkaline water; buffered saline is preferred because it provides a cleaner background and better preservation of parasite morphology. Although most people do not filter the working stain prior to use (if using a Coplin jar), results are better overall if the working stain is filtered through Whatman no. 1 filter paper immediately before use. Make sure to use absolute methanol (acetone-free) for fixing thin blood films. Stock buffered water is stable for 1 year at room temperature. Working stock buffered water is stable for 1 month at room temperature. Stock Giemsa stain is stable at room temperature indefinitely; stock stain appears to improve with age (similar to what is seen with iron-hematoxylin stains). A 45- to 60-min staining time appears to work better than 15 min; staining times will depend on stain dilution. Some workers feel the use of a 10% Triton solution is helpful, while some feel it is detrimental to the overall quality.

12. How should you handle a delay between thick film preparation and staining?

Thick films can be preserved, particularly if there will be a delay prior to staining.

Dip the slides in a buffered methylene blue solution (0.65% for 1 to 2 s and allow the slides to dry.

Methylene blue	0.65 g
Disodium hydrogen phosphate Na_2HPO_4	2.0 g
Potassium dihydrogen phosphate KH_2PO_4	0.65 g
Distilled water	1.0 liter

Mix and store in a brown-stoppered bottle.

The benefits of such an approach are as follows:

a. The RBCs are hemolyzed.
b. Color is introduced into the cytoplasm of the parasites.
c. Prestaining with methylene blue helps preserve the organisms on the smear.

13. Can blood stains other than Giemsa stain be used to stain the blood films?

The detection of parasites in the blood has been made possible by the use of Romanovsky-type differential stains which selectively color the nuclear material red and the cytoplasm blue. This reaction takes place under optimal pH conditions: rather than using a neutral pH, optically perfect results are obtained using the slightly basic pH of 7.2 (due to the optical dominance of red pigmentation). Although for many years, Giemsa stain has been the stain of choice, the parasites can also be seen on blood films stained with Wright's stain, a Wright/Giemsa combination stain, or one of the more rapid stains such as Diff-Quik (American Scientific Products, McGraw Park, IL), Wright's Dip Stat Stain (Medical Chemical Corp., Torrance, CA), or Field's stain. **It is more appropriate to use a stain with which you are familiar, rather than Giemsa, which is somewhat more complicated to use. PMNs will serve as the quality control (QC) organism for any of the blood stains. Any parasites present will stain like the PMNs, regardless of the stain used.** Also, the CAP checklist does not mandate the use of Giemsa stain.

14. How should malaria blood films (both thick and thin films) be examined?

A minimum of 300 oil immersion fields using the 100× objective should be examined. The blood film can be scanned using a 50× or 60× oil immersion lens, but final reporting of the results should be based on the use of the 100× oil immersion lens for a total magnification of ×1,000.

15. What type of QC slides should be used for blood parasite work?

Regardless of the stain you are using (Giemsa, Wright, Wright/Giemsa, rapid stains), **your QC slide is the actual slide you are staining.** This approach to QC is acceptable to CAP, as well. **Any parasites present will stain like WBCs, so your QC is built into the system.**

16. What is a good source for teaching slides?

A good source for teaching slides is Meridian Bioscience, Inc. They have slides and specimens available for sale. Contact them for a brochure: (800) 543-1980, ext. 335.

ORGANISM IDENTIFICATION (1–4; this volume)

1. Why is it so important to "rule out" infections with *Plasmodium falciparum* or *Plasmodium knowlesi*?

P. falciparum causes more serious disease than the other three species (*P. vivax*, *P. ovale*, *and Plasmodium malariae*). *P. falciparum* tends to invade all ages of RBCs, and the proportion of infected cells may exceed 50%. **Also remember that infections**

with *P. knowlesi* are considered as serious as those with *P. falciparum* (24-hour life cycle, heavy parasitemia, all ages of RBCs). Schizogony occurs in the internal organs (spleen, liver, bone marrow, etc.) rather than in the circulating blood. Ischemia caused by the plugging of vessels within these organs by masses of parasitized RBCs will produce various symptoms, depending on the organ involved.

Onset of a *P. falciparum* malaria attack occurs from 8 to 12 days after infection and is preceded by 3 to 4 days of vague symptoms such as aches, pains, headache, fatigue, anorexia, or nausea. The onset is characterized by fever, a more severe headache, and nausea and vomiting, with occasional severe epigastric pain. There may be only a feeling of chilliness at the onset of fever. Periodicity of the cycle will not be established during the early stages, and the presumptive diagnosis may be totally unrelated to a possible malaria infection. If the fever does develop a synchronous cycle, it is usually a cycle of somewhat less than 48 hours. An untreated primary attack of *P. falciparum* malaria usually ends within 2 to 3 weeks. True relapses from the liver do not occur, and after a year, recrudescence is rare. **Severe or fatal complications of *P. falciparum* malaria can occur at any time during the infection and are related to the plugging of vessels in the internal organs, the symptoms depending on the organ(s) involved.** Note: The primary objective when performing blood film examination for parasites is to rule out *P. falciparum* or *P. knowlesi*. It is also becoming more widely recognized that *P. vivax* can cause severe sequelae; this infection is not nearly as benign as previously thought.

2. What are some of the problems associated with the differentiation between *Plasmodium* spp. and *Babesia* spp.? Can *Babesia* spp. cause serious infections?

The ring forms of all four species of *Plasmodium* can mimic the ring forms of *Babesia* spp. Multiple rings per cell are more typical of *P. falciparum* than of the other four species that cause human malaria. *Babesia* rings are often numerous, of smaller sizes, and tend to be very pleomorphic, while those of *P. falciparum* tend to be fewer in number and more consistent in size and shape. Remember that *P. falciparum* rings may appear somewhat larger if the blood has been drawn in EDTA and there is any lag time prior to thick and thin film preparation. Differentiation between *Plasmodium* spp. and *Babesia* spp. may be impossible without examining a thin blood film (rather than a thick blood film). Often, the parasitemia in a *Babesia* infection may be heavier than that seen with *P. falciparum*, particularly when a patient presents early in the infection. It is also important to remember that some of the *Babesia* spp. can cause severe illness (*Babesia divergens* from Europe, etc. [42% mortality], *Babesia* from California, Oregon, Washington, Missouri), while some infections such as those caused by *Babesia microti* cause less serious or subclinical infections. **However, any of the *Babesia* spp. can cause severe disease in immunocompromised patients,** particularly those patients who have undergone splenectomy.

3. What is the significance of finding only ring forms on two sets of blood films drawn 6 hours apart?

Remember that all of the life cycle stages (rings, developing trophozoites, early schizonts, late schizonts, mature schizonts, and gametocytes) can be seen on the blood films in infections with *P. vivax, P. ovale, P. malariae*, and *P. knowlesi*. Due to unique characteristics of the life cycle, only rings and gametocytes (and occasional mature schizonts) are seen in the peripheral blood with a *P. falciparum* infection. **Therefore, if you see two sets of blood films (collected 6 hours apart) that contain ring forms only, there is an excellent chance the patient is infected with *P. falciparum*, the most serious of the five *Plasmodium* spp. infections.**

4. Why aren't gametocytes of *P. falciparum* seen in many patients presenting to the emergency room (ER)?

Many patients present to the ER early in the infection prior to the formation of the gametocytes; thus, the diagnosis must be made based on seeing the ring forms only. As you can imagine, identification of *Plasmodium* to the species level can be very difficult when only rings are present. These patients tend to be travelers who have had no prior exposure to *P. falciparum* (immunologically naive) and who become symptomatic very early after being infected. It normally takes approximately 10 days for the crescent-shaped *P. falciparum* gametocytes to form.

5. What should be considered if the patient has been diagnosed with a *P. falciparum/P. malariae* mixed infection, particularly with a travel history to Malaysia?

P. knowlesi mimics *P. falciparum* early in the infection, while the morphology mimics *P. malariae* later in the cycle. Although relatively uncommon, infections with the fifth human malaria, *P. knowlesi*, are being seen in other parts of the world, primarily in travelers who have been to Malaysia.

REPORTING

1. Why is it important to identify malaria organisms to the species level?

Since *P. falciparum* and *P. knowlesi* can cause severe disease and death, it is very important for the physician to know whether these infections can be ruled out. It is also important to know if any of the other three species are present, particularly *P. vivax* or *P. ovale*, which would require therapy for both the liver and RBC stages due to potential relapse from the liver stages. It is also important because of potential drug resistance (chloroquine, *P. falciparum*, *P. vivax*; primaquine tolerance/resistance, *P. vivax* [rare, but documented]).

2. How should a positive malaria blood film be reported?

A. Using the thin blood film method, report the percentage of parasite-infected RBCs per 100 RBCs counted.

Example: *P. falciparum*, parasitemia = 0.01%

B. Using the thick/thin blood film method, report the number of parasites per microliter of blood.

Example: *P. falciparum*, parasitemia = 10,000/µl of blood

3. How should results be reported if *Plasmodium* spp. parasites are seen, but *P. falciparum* or *P. knowlesi* infection cannot be ruled out?

It is important to convey to the physician that *P. falciparum* or *P. knowlesi* cannot be ruled out; therapy may be initiated on the assumption that this species might be present. The report should read:

Plasmodium spp. present; unable to "rule out" *P. falciparum* or *P. knowlesi.*

4. How often do mixed infections occur, and how should they be reported?

It is important to remember that mixed infections are much more common than suspected and/or reported. When rings are present, along with other developing stages (*P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*), always look for the presence of two populations of ring forms, one of which might be *P. falciparum*. The report should read:

Plasmodium spp. present, possible mixed infection; unable to "rule out" *P. falciparum* or *P. knowlesi.*

Another report example might be:

Plasmodium vivax rings, developing schizonts, and gametocytes; possible mixed infection: unable to "rule out" *P. falciparum* or *P. knowlesi.*

5. Why is it important to report the *Plasmodium* spp. stages seen in the blood films?

Often patients who have been diagnosed with *Plasmodium* spp. and are not suspected of having drug-resistant malaria (*P. falciparum*, *P. vivax*) are treated with chloroquine. Chloroquine will not eliminate any gametocytes present, and there are mosquitoes within the United States that can transmit malaria if they take a blood meal from an individual with gametocytes in the blood. Thus, it is important for the physician to know which stages are present in the blood (rings, developing trophs, schizonts, and/or gametocytes).

6. How do parasitemia and malaria severity correlate?

The following percentages are helpful in interpretation of malaria severity:

- Parasitemia of >10,000/µl = heavy infection
- 0.002%, 100/µl, symptomatic below this level
- 0.2%, 10,000/µl, immune patients symptomatic
- 0.1%, 5,000/µl, sensitivity of rapid malaria test (BinaxNOW) at close to 100%; will decrease significantly if parasitemia is below 0.1%
- 2%, 100,000/µl, maximum parasitemia for *P. vivax, P. ovale*; rarely go above 2%
- 2 to 5%, up to 250,000/µl, severe malaria, mortality
- 10%, 500,000/µl, exchange transfusion

PROFICIENCY TESTING

1. How should blood films be examined for proficiency testing (PT)?

Since you have no idea what organisms might be present, always review the blood films using the 10× objective (**entire** slide). This examination is likely to reveal any microfilariae that are present; however, small parasites such as *Plasmodium* and *Babesia* may be missed. Before reporting the smear as negative, examine at least 300 oil immersion fields using the 100× oil immersion objective.

2. Do PT blood films match those seen from actual patients?

Yes and no. Blood films for PT are actual patient specimens, but they may have a higher parasitemia than is seen in many patients reporting to the ER, clinic, etc. Often, smears contain a higher parasitemia than is commonly seen. So the PT smears represent a mix, some of which are fairly typical and some of which have a high number of organisms present. **As in the case of a traveler, the thin blood film may appear to be negative, while the thick film will be positive. In a clinical setting, these blood film results are not that unusual, particularly in an immunologically naive patient (traveler with no prior exposure to *Plasmodium* spp.).**

3. What blood parasites might be seen in PT specimens?

The following parasites may be seen in PT specimens: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi* (less likely) mixed malaria infections, *Babesia* spp., *Trypanosoma cruzi*, *Trypanosoma rhodesiense/gambiense*, *Leishmania* spp., and microfilariae.

GENERAL QUESTIONS

1. What stains are recommended for staining microfilariae?

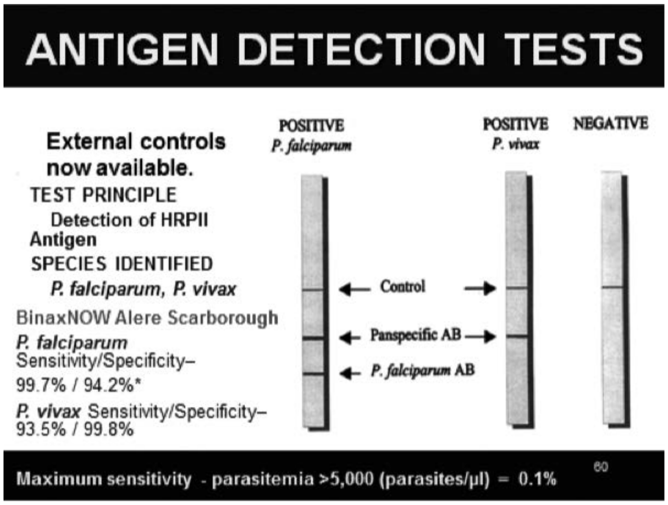
Although Giemsa stain is generally recommended, it will not stain the sheath of *Wuchereria bancrofti*; hematoxylin-based stains (Delafield's) are recommended. Also, the sheath of *Brugia malayi* will stain pink with Giemsa stain.

2. What other methods can be used for the identification of blood parasites?

- Concentrations (trypanosomes, microfilariae, leishmaniae)
- Buffy coat thick and thin blood films (all blood parasites, including *Plasmodium* spp.)
- Skin biopsies or bone marrow aspirates (Giemsa or other blood stains) (leishmaniae)
- Rapid methods/dipstick formats (malaria, microfilariae) (Fig. A9.4; see also Table A9.3). **Note: The Alere/Binax rapid test for *Plasmodium falciparum* and *Plasmodium* spp. is FDA approved (June 2007); a positive external control is also available for customers.** The Binax rapid test is as sensitive as a good microscopist, but often these skilled individuals may not be available on off-hour shifts. However, it is important to recognize the pros and cons of the rapid test when compared to microscopic examination of blood films, particularly in travelers in whom the parasitemia may be considerably less than 0.1%.

3. How good are serologies for malaria; when should they be performed?

Malaria serologies are available through the Centers for Disease Control and Prevention (CDC). They always test with antigens of all five species, but the reactions are not species specific; there is a lot of cross-reactivity. *Plasmodium* spp. can be determined in about 80% of **nonimmune** patients in their **primary** infection (U.S. Army during Vietnam War), but species cannot be determined in patients with long-term exposure and multiple infections (i.e., Africans). The CDC does not recommend serology for identification to the species level; PCR is the best tool for this purpose if pretreatment EDTA blood is available.



ANTIGEN DETECTION TESTS

External controls now available.

TEST PRINCIPLE

Detection of HRPII Antigen

SPECIES IDENTIFIED

P. falciparum, P. vivax

BinaxNOW Alere Scarborough

P. falciparum Sensitivity/Specificity– 99.7% / 94.2%*

P. vivax Sensitivity/Specificity– 93.5% / 99.8%

Maximum sensitivity - parasitemia >5,000 (parasites/µl) = 0.1%

Figure A9.4 General diagram of a rapid malaria test. Readings: top ("Control"), the negative test shows the control line only; middle ("Panspecific AB"), control line plus the *P. vivax* line indicates the presence of a panspecific antigen (common to all *Plasmodium* spp. but most sensitive for *P. vivax* rather than *P. ovale* and *P. malariae*); bottom ("*P. falciparum* AB"), control line, panspecific antigen line, and line specific for *P. falciparum* antigen presence. Note: most cartridge rapid malaria tests have not been developed to detect *P. knowlesi* (under development). See Table A9.3. doi:10.1128/9781555819002.Apx9.f4

4. How do you categorize malaria resistance (seen in both *P. falciparum* and *P. vivax*)?

See Table A9.4.

5. How soon after initiation of therapy for *P. vivax* malaria would one expect to see clearance in blood smears? How soon should follow-up blood smears be submitted for evaluation after therapy?

Although the number of cases we see in the United States is certainly less than in areas of endemicity, we begin to see a drop in parasite numbers very quickly (within a few hours) after the initiation of therapy. If an individual presents in the ER, the parasitemia is often below 1 to 2% (0.1 to 0.01%); the patient is given therapy and is not admitted to the hospital (in the case of *P. vivax*, *P. malariae*, or *P. ovale*). If the organism is identified as *P. falciparum*, *P. knowlesi*, or a mix (most common would be *P. falciparum*/*P. vivax* or *P. malariae*), the patient will be admitted. In some cases, we do not receive blood for follow-up examination (from ER patients where *P. falciparum* and *P. knowlesi* have been ruled out). When treating *P. falciparum*, we receive blood samples approximately every 4 to 6 hours for routine checks (patients will be admitted to the hospital for treatment). Although the number of resistant *P. vivax* cases is small, it has been confirmed, so this is always a consideration, as are mixed infections with *P. falciparum* and *P. vivax*.

6. Where can one get PCR performed for the malaria diagnosis and identification to the species level?

The CDC performs PCR for malaria, which identifies the organisms to the species level. The CDC requires about 0.5 ml of pretreatment EDTA blood. Also, if possible, they like to review both thick and thin smears if extra blood films are available. Specimens can be sent overnight to:

Microbiologist
Division of Parasitic Diseases
Centers for Disease Control and Prevention
Building 109, Room 1302
4770 Buford Highway NE
Atlanta, GA 30341
Phone: 770-488-7044

7. What is the current status of rapid testing for malaria?

This issue has been a difficult one to resolve in the United States. Currently, the Binax rapid test was approved by the FDA in June 2007 (Fig. A9.4) (see Table A9.5). An external positive control is also available for customers. Certainly the use of these rapid tests would be a tremendous advantage for personnel on the night shift. However, **if the test is negative, it would not rule out a *Plasmodium* spp. infection, and thick and thin blood films would need to be prepared and examined STAT.** If positive, then follow-up would need to be performed on slides to rule in/out mixed infections. All published information relevant to these kits indi-

TABLE A9.3 Sensitivity of the Alere BinaxNOW rapid test[a]

Organism	Parasitemia level	% Sensitivity (positive/total)	95% CI
P. falciparum	<5,000	99.7 (326/327)	98–100%
	1,000–5,000	99.2 (126/127)	96–100%
	500–1,000	92.6 (25/27)	76–99%
	100–500	89.2 (33/37)	75–97%
	0–100	53.9 (21/39)	37–70%
Overall		95.3 (531/557)	93–97%
P. vivax	>5,000	93.5 (462/494)	91–96%
	1,000–5,000	81.0 (277/342)	76–85%
	500–1,000	47.4 (37/78)	36–59%
	100–500	23.6 (34/144)	17–31%
	0–100	6.2 (8/129)	3–12%
Overall		68.9 (818/1,187)	66–72%

[a] Parasitemia level of 5,000 = 0.1%. CI, confidence interval.

TABLE A9.4 Categories of malaria resistance

Resistance definition	Comments
Sensitive	From initiation of therapy, asexual parasites are cleared by day 6; no evidence of recrudescence up to day 28. Peripheral blood films appear to go from positive to negative very quickly (can be a change from one draw to the second draw 6 hours later).
Resistance type I (RI)	From initiation of therapy, asexual parasites have cleared for at least 2 consecutive days (the latest day being day 6); recrudescence follows. Parasite count initially drops, and blood films appear to be negative; patient should be monitored for a period of days, particularly if drug-resistant *P. falciparum* is suspected.
Resistance type II (RII)	Within 48 hours of initiation of therapy, marked reduction of asexual parasitemia to <25% of pretreatment count; however, no subsequent disappearance of parasitemia (smear positive on day 6). Patient appears to be improving; parasite count drops, but blood films always appear positive.
Resistance type III (RIII)	Modest reduction in parasitemia may be seen; no change or increase in parasitemia seen during first 48 hours after treatment; no clearing of asexual parasites. In some cases, the parasite count continues to increase with no visible decrease at any time; blood films show overall parasite increase.

cates that they are diagnostically helpful, but they are no better and somewhat less accurate than a good microscopist. However, as we all know, these individuals are often not available during off-hour shifts. Many labs have solved the problem by bringing someone in just to prepare and read slides during off hours. Often, the PM shifts prepare and stain smears (Wright-Giemsa or one of the rapid stains) and another individual examines the thick and thin stained blood films. We tried to expose our residents, postdocs, etc., to these kits and found them to be helpful. **However, when dealing with immunologically naive patients with very low parasitemia values, it would be easy to miss an infection unless careful examination of the thick films was performed. Remember, the rapid test will not take the place of careful examination of both thick and thin blood films.** In other parts of the world, a number of rapid malaria tests are available (15).

8. Can mosquitoes be infected with more than one species of malaria?

There appears to be no barrier to infection of *Anopheles* with mixed *Plasmodium* species. Mosquitoes doubly infected with *P. falciparum* and *P. vivax* were able to transmit both species to humans following deliberate feeding on volunteers (16). In natural situations, however, suppressive effects in the human host may lead to overlapping waves of gametocytes of different species so that there is a tendency for mosquitoes to be infected with only one species. In some cases there may be specific suppression of sporogony of one species compared to the other. For example, in areas where *P. falciparum* and *P. malariae* are sympatric, the cold temperatures associated with altitude may be disadvantageous to *P. falciparum*, which requires a higher temperature

TABLE A9.5 Parasitemia definitions

% Parasitemia	Comments
Parasitemia of >10,000/µl = heavy infection	
0.0001–0.0004, 5–20/µl	Required for a positive thick blood film
0.002%, 100/µl	Immunologically naive patients may be symptomatic below this level
0.02%, 1,000/µl	Can be seen in travelers in ERs (symptomatic very early with low parasitemia = immunologically naive)
0.1%, 5,000/µl	**BinaxNOW rapid lateral flow method** = level of sensitivity below this percentage drops significantly; problem with travelers (0.01% or less)
0.2%, 10,000/µl	Immune patients are symptomatic
2%, 100,000/µl	Maximum parasitemia for *P. vivax* and *P. ovale*; rarely goes >2%
2–5%, up to 250,000/µl	Severe malaria, high mortality
10%, 500,000/µl	Exchange transfusion usually required

for sporogony than *P. malariae*. This may lead to seasonal changes in the prevalence of the two species.

9. What is the general status of PCR for leishmaniasis?

PCR for leishmaniasis has gone through some very rigorous validation at the leishmaniasis lab at Walter Reed Army Institute of Research and is excellent. Contact Dr. Glen Wortmann at glenn.wortmann@us.army.mil.

10. Are serologies available for the African trypanosomes?

The CDC doesn't do PCR for African trypanosomiasis. It is unnecessary for diagnosing *T. b. rhodesiense* (parasites easily detectable by microscopy). PCR for *T. b. gambiense* would need to be arranged by a laboratory in Europe. It is viewed as a research tool only. The CDC consultant is Dr. Anne Moore (770-488-7776).

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APPENDIX 10

Current Procedural Terminology (CPT) Codes in Parasitology

Procedure name[a]	CPT
Blood Film (Thick), Blood film stain (includes concentration)	87015 + 87207
Blood Film (Thin), Blood film stain	87207
Buffy Coat Blood Film (Thick), Blood film stain (includes concentration)	87015 + 87207
Buffy Coat Blood Film (Thin), Blood film stain (includes concentration)	87015 + 87207
Calcofluor Stain (direct material, no concentration)	87206
Calcofluor Stain (concentrated material)	87015 + 87206
Concentration, Baermann (plus Wet Mount Examination)	87015 + 87210
Concentration, Blood (Concentration, Wet Mounts) (may be multiple wet mounts prepared/examined—use additional 87210 with modifier) (will include Knott and Membrane Concentrations using Nuclepore filters)	87015 + 87210
Concentration, Harada-Mori (plus Wet Mount Examination)	87015 + 87210
Concentration, Iodine Prep (Wet Prep only)	87015 + 87210
Concentration (Miscellaneous)	87015
Concentration, Petri Dish (plus Wet Mount Examination)	87015 + 87210
Concentration, Saline Prep (Wet Prep only)	87015 + 87210
Counterimmunoelectrophoresis, each antigen (example: *Entamoeba histolytica*)	86185
Cryptosporidium Examination (Modified AFB) (Concentration Sediment)	87015 + 87207
Cryptosporidium Examination (FA) (Concentration Sediment)	87015 + 87272
Cryptosporidium Examination (EIA) (Plate/Tray)	87328
Cryptosporidium Examination (Cartridge)	87328[b]
Cryptosporidium/Giardia Examination (FA) (Concentration Sediment)	87015 + 87272 + 87269
Cryptosporidium/Giardia Examination (EIA) (screen that detects both, but does not differentiate between the two; if positive, separate reagents must be used)	87328 + 87329 (Neg)
Cryptosporidium/Giardia/Entamoeba histolytica/E. dispar group, Cartridge	87328 + 87329 + 87336
Cyclospora Examination (Modified AFB) (Concentration Sediment)	87015 + 87207
Cyclospora Examination (Autofluorescence) (Concentration Sediment)	87015 + 87210
Culture (*Acanthamoeba*) (Agar plates) (Screen, Wet Mount, Permanent Stain) (trichrome, use 87209)	87081 + 87210 + 87207 (Giemsa)
Culture (Intestinal and Blood Protozoa) (Culture, Concentration, Wet Mount, Permanent Stain of culture sediment)	87081 + 87215 + 87210 + 87207 (Giemsa) or 87209 (Trichrome)

Culture, Agar Plate (*Strongyloides*)	87081 + 87210
Culture (*Trichomonas*) (Pouch) (Screen) (microscope exam through plastic pouch)	87081
Ectoparasite Identification (Macro) (Arthropod)	87168
Ectoparasite Identification (Wet Mount) (Arthropod)	87210
Ectoparasite Identification (Skin, Scabies)	87220
Entamoeba histolytica/E. dispar Group (EIA)	87336
Entamoeba histolytica (EIA)	87337
Examination, Wet Prep Only (Wet Prep only)	87210
Examination, Duodenum (Concentration, Wet Mounts)	87177
Examination, Duodenum (Permanent Stain)	87209
Examination, EnteroTest (Wet Mounts only)	87210
Examination, EnteroTest (Permanent Stain only)	87209
Examination, Urine (Random) (Concentration, Wet Mounts) (multiple wet mounts may be prepared/examined—use additional 87210 with modifier)	87015 + 87210
Examination, 24 h Urine (Concentration, Wet Mounts) (multiple wet mounts may be prepared/examined—use additional 87210 with modifier)	87015 + 87210
Fat, Stool (Qualitative)	82705
Giardia Examination (FA) (Concentration Sediment)	87015 + 87269
Giardia Examination (EIA) (Plate/Tray)	87329
Giardia Examination (Cartridge)	87329[b]
Helminth Egg Hatching (Concentration, Wet Mounts)	87177
Isospora (*Cystoisospora*) Examination (Modified AFB) (Concentration Sediment)	87015 + 87207
Leishmania Lesion, Stained Smear	87207
Microsporidia, Special Stain (Modified Trichrome) (on concentration sediment)	87015 + 87207
Microsporidia, Fluorescent Stain (Calcofluor) (on concentration sediment)	87015 + 87206
Occult Blood, Stool	82270
Occult Blood, Gastric	82273
Ova and Parasite Examination (Direct Smears, Concentration and Smears) (also recommended for Concentration and Wet Mounts)	87177
Ova and Parasite Examination (Permanent Stain) (example: trichrome or iron hematoxylin)	87209
Ova and Parasite Examination (Complete Examination) (Direct Smears, Concentration, Permanent Stain)	87177 + 87209
Petri Dish Culture/Concentration (plus Examination)	87015 + 87210
pH, Body Fluid except blood (Stool)	83986
Pinworm Examination, Cellophane Tape	87172
Plasmodium (Parasitemia Determination) Manual Cell Count (RBCs, WBCs, **OR** platelets) each (slide preparation and stain—add separate codes)	85032
Plasmodium (Rapid Tests—dipstick, etc.), infectious agent **antigen** detection by immunoassay with direct optical observation, not otherwise specified T1 = *P. falciparum*; T2 = Pan-malaria band; T3 = Internal control	87899[b] × 3
Reducing Substances, Stool (pH)	83986
Referral In (Stool for Ova and Parasite Exam)	87177 + 87209
Tissue Homogenization for culture	87176
Trichomonas vaginalis, Direct Probe (example: Affirm)	87660
Trichomonas vaginalis, Detection Immunoassay with direct optical observation (example: Osom)	87808
Trypsin Activity, Stool (Qualitative)	84488
Worm Identification (Macro)	87169
Worm Identification (Wet Mount)	87210

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[a] EIA, enzyme immunoassay; FA, fluorescence assay; AFB, acid-fast bacilli; RBCs, red blood cells; WBCs, white blood cells.

[b] Because there is no analyte code for *Cryptosporidium* or *Giardia* for "immunoassay with direct optical observation" (cartridges), it is appropriate to use the analyte specific code for EIA for the two organisms (for cartridge formats). Since there is a specific code for *Trichomonas* "immunoassay with direct optical observation," this number should be used. **In the coding hierarchy, analyte comes before method.** In the case of the rapid

Plasmodium tests (dipsticks, cartridges), there is no analyte listed for EIA, so in these cases we need to use the nonspecific code: "Infectious agent **antigen** detection by immunoassay with direct optical observation, not otherwise specified" (87899).

NOTES:

When using FA reagents or special stains for the coccidia or microsporidia, you should always perform these tests on centrifuged material, thus the need to add the concentration code 87015.

For multiple specimens or sites per patient per day, use modifier "-91"

> Example: blood for malaria (receipt of more than one specimen/patient/day). This is a repeat of the same test to increase analytical sensitivity.

For repeat laboratory tests performed on the same day/patient, use modifier "-91"

> Example: multiple wet preparation exams for duodenal aspirate (examination of more than one wet preparation from a single specimen per patient per day).

For multiple stool specimens collected on different days, but submitted to the laboratory on one day, code for separate dates (no modifier required).

KEY POINT

When considering a potential audit situation, remember that THE TEST CPT CODES AND BILLS SHOULD MATCH THE SAME TESTS AND BILLS FOR THE TESTS THE PHYSICIAN ORDERED. THEREFORE, IT IS CRITICAL THAT YOUR REQUISITION FORMS AND/OR COMPUTER ORDER SCREENS MATCH WHAT YOU ARE ACTUALLY CODING AND BILLING. This is now an issue since *Giardia* and *Cryptosporidium* (FA, EIA) have now been broken out and should be coded and billed as separate tests.

CPT codes, parasitology, revision 2007 (Immunology, **Antibody** Testing)

Procedure name	CPT
Antibody Detection	
Giardia lamblia	86674
Helminth, not elsewhere specified	86682
Leishmania	86717
Plasmodium	86750
Protozoa, not elsewhere specified (example: *Entamoeba histolytica*)	86753
Toxoplasma	86777
Toxoplasma, IgM	86778
Trichinella	86784
Complement Fixation	
Echinococcosis	86171
Trypanosomiasis	86171
Hemagglutination Inhibition Test (HAI)	
Echinococcosis	86280
Filariasis	86280
Trypanosomiasis	86280
Immunoassay for infectious agent **antibody**, qualitative or semiquantitative, single-step method (e.g., Reagent strip)	86318

Glossary

Certain terms listed here do not appear in the text, but it is hoped that their inclusion will be useful. Diseases and parasites in parentheses represent some, but not all, examples that pertain to the terms listed. One of my favorite definitions is "Parasitologist: A quaint person who seeks truth in strange places" (Schmidt GD, Roberts LS, *Foundations of Parasitology*, CV Mosby Co, St. Louis, MO, 1977).

Abdominal pain Crampy abdominal pain that may be seen in amebic colitis; right upper quadrant pain in amebic abscess; severe duodenitis or jejunitis with *Strongyloides* organisms penetrating the mucosa; pain suggestive of gastric ulcer or appendicitis with anisakid larvae (penetration of gut wall).

Aberrant host One in which the parasite cannot complete its development or appropriate phase of its life cycle.

Aberrant parasite One which is never transmitted from person to person and which develops abnormally in humans (*Echinococcus multilocularis*, *Angiostrongylus*).

Abopercular end In helminth eggs that have an operculum, the opposite end is the abopercular end.

Abscess A localized collection of pus caused by liquefaction necrosis of tissue (amebiasis, filariasis).

Acanthamoebiasis Infection by free-living soil and water amebae of the genus *Acanthamoeba* that may result in a necrotizing dermal or tissue invasion, a fulmination and usually fatal primary amebic meningoencephalitis (PAM), or a subacute or chronic granulomatous amebic encephalitis (GAE).

Acaricide A mite-destroying agent.

Accidental host A host in which the parasite is not commonly found, but which is suitable for the parasite's development. In some instances (e.g., cysticercosis) the accidental host becomes a "dead end" because even though the parasite develops through its appropriate stages, it fails to find a portal of exit and is thus blocked from continuing its life cycle.

Accidental parasite A parasite found in other than its normal host; also called an incidental parasite.

Accolé Early ring form of *Plasmodium falciparum* found at the margin of a red cell: appliqué.

Acetabulum A muscular attachment organ, "sucker" found in helminths.

Acid-fast stain, modified Developed for staining some of the coccidia (*Cryptosporidium*, *Cyclospora*); must be used with gentle decolorizer (1% sulfuric acid recommended).

Acquired immunity Immunity arising from a specific immune response, humoral or cell mediated, stimulated by antigen in the body of the host (active) or in the body of another individual with the antibodies or lymphocytes transferred to the host (passive).

Acute abdomen An abdominal condition of abrupt onset usually associated with abdominal pain resulting from inflammation, perforation, obstruction, infarction, or rupture of intra-abdominal organs; surgical intervention is usually necessary (ascariasis, anisakiasis).

Aerosol A system of respirable particles dispersed in a gas, smoke, or fog that can be retained in the lungs.

AIDS Acquired immune deficiency syndrome.

Alae Pronounced, longitudinal cuticular ridges in nematodes, usually found in larval stages (*Ascaris lumbricoides*), although occasionally present in adult worms (*Enterobius vermicularis*).

Allergy A hypersensitive condition acquired by exposure to a particular allergen (helminth infections, house dust mites, ectoparasite bites or stings).

Amastigote Small, round, intracellular stage of *Leishmania* spp. and *Trypanosoma cruzi* in which the base of the flagellum is anterior to the nucleus but there is no external flagellum; also called Leishman-Donovan body, L-D body, or leishmanial stage.

Anamnestic response Immune response to a challenge or secondary antigen inoculation, marked by a more rapid and stronger manifestation of the immune reaction than after the primary immunizing dose.

Anaphylaxis Hypersensitivity produced by exposure to further doses of the same protein, usually when exposure is within less than 2 weeks (bee stings, echinococcosis).

Anemia A reduced number of erythrocytes per cubic millimeter, reduction in the amount of hemoglobin, or reduction in the volume of packed red blood cells per 100 ml of blood (malaria, hookworm, and *Diphyllobothrium latum* infections).

Anergic Absence of sensitivity to an antigen or the condition resulting from desensitization (cutaneous leishmaniasis).

Aneurysm Circumscribed dilation of an artery connecting directly with the lumen of an artery or a cardiac chamber connecting directly with the lumen of an artery, usually due to an acquired or congenital weakness of the wall of the artery or chamber.

Anorexia Absence of appetite.

Anthropophilic Human-seeing or human-preferring, especially with reference to blood-sucking arthropods.

Anthropozoonosis A zoonosis maintained in nature by animals and transmissible to humans.

Antibodies Proteins (immunoglobulins) found in blood or other body fluids; produced in response to foreign antigens; produced by B cells.

Antigenic determinant The areas on an antigen molecule that bind with antibody or specific receptor sites on the sensitized lymphocyte; these areas determine the specificity of the antibody or lymphocyte.

Antigenic variation An immune evasion strategy used by various parasites; surface antigens are altered from one generation to the next, often as a result of antibody development. (*Trypanosoma* spp.).

Anuria Absence of urine secretion (scorpion sting).

Aphasia Partial or complete inability to speak and/or understand spoken words.

Apoptosis Single deletion of scattered cells by fragmentation into membrane-bound particles, which are phagocytosed by other cells; believed to be due to programmed cell death.

Appendicitis Inflammation of the vermiform appendix (amebiasis, *Ascaris*, *Trichuris*, and *Enterobius* infections).

Appendix Thick-walled fingerlike projection at the end of the cecum.

Appliqué Early ring form of *Plasmodium falciparum* found at the margin of a red cell; accolé.

Artemisinin-based combination therapy A standard antimalarial drug regimen in which artemisinin is taken in combination with one or more other antimalarial drugs.

Ascites Effusion of serous fluid into the abdominal cavity (schistosomiasis).

Aspirate Fluid removed from a cavity or lesion (leishmaniasis, hydatid disease, amebiasis).

Asthma, bronchial Disease characterized by wheezing, coughing, and difficulty in breathing caused by spasmodic contraction of the bronchi; may be the result of allergic reaction (ascariasis, strongyloidiasis, visceral larva migrans, house dust mites). *See* Eosinophilia.

Autochthonous Indigenous or normally found in a particular area.

Autoinfection Reinfection by an organism already present in the body with an increase in the number of parasites without their undergoing a cycle outside the body; self-infection (*Strongyloides stercoralis*, *Hymenolepis nana*, *Cryptosporidium* spp.).

Autopsy Gross and microscopic postmortem examination of the organs of the body to determine the cause of death or pathologic changes.

Avidity Multiple bond interactions; used to describe antibody-antigen interactions; multiple bond binding strength.

Axoneme In flagellates, an internal fibril arising from a blepharoplast and passing through the cytoplasm. An axoneme may leave the body of the flagellate with a small sheath of cytoplasm to become a flagellum or run along the surface of the body lifting the periplast (cell membrane) to form an undulating membrane.

Axostyle Rodlike supporting structure in some flagellates that gives rigidity to the body (*Trichomonas* spp.).

Babesiosis A disease of humans that is caused by a blood protozoan of various animals; tick borne; organism morphology similar to young rings of *Plasmodium falciparum*; more severe in compromised patients.

Band form Older trophozoites of *Plasmodium malariae* and *Plasmodium knowlesi* that may stretch across the red cell in the form of a band.

Barbiero Brazilian term for the blood-sucking hemipteran triatomid bug, *Panstrongylus megistus*, an important vector of Chagas' disease (caused by *Trypanosoma cruzi*).

Basket nucleus Nuclear structure that may be seen in *Iodamoeba bütschlii* cysts (occasionally trophozoites); in well-stained organisms, fibrils may be seen running between the karyosome and the chromatin granules. The "basket of flowers" has been described as follows: the karyosome forms the basket, the fibrils form the stems, and the granules form the flowers.

Baylisascaris A genus of ascarid nematodes found in the intestines of mammals, including humans.

Baylisascaris procyonis A common roundworm of raccoons (ingestion of infective eggs from the environment) that can cause larva migrans (central nervous system). Human cases present with eosinophilic meningoencephalitis (there have been several fatal cases in the United States).

Behçet's syndrome More common in men; involves dermatitis, erythema nodosum, thrombophlebitis, cerebral involvement; may include arthritis, uveitis, or iridocyclitis with hypopyon.

Benign tertian malaria Malaria caused by *Plasmodium vivax*.

Bilharziasis Another name for schistosomiasis; often used to indicate infection with either *Schistosoma mansoni* or *Schistosoma haematobium*.

Binary division (fission) Dividing of a cell into two nearly equal daughter individuals.

Biopsy Removal of tissue from a living person; examination, usually microscopic, used to make diagnosis (Chagas' disease, leishmaniasis, schistosomiasis, visceral larva migrans, amebiasis).

Blackwater fever A condition in which the diagnostic symptom is the passage of reddish to black urine (containing hemoglobin), indicating massive intravascular hemolysis (*Plasmodium falciparum*).

Blepharoplast A small, dark-staining body near the base of the flagellum that is associated with fibrillar portions of the flagellum (blood flagellates).

Botfly Robust, hairy fly of the order *Diptera*, often strikingly marked in black and yellow or gray, whose larvae produce a variety of myiasis conditions in humans and various domestic animals.

Bothrium One of the two shallow grooves on the scolex of *Diphyllobothrium latum*.

Bots The larvae of several species of botflies.

Bradyzoite A slowly multiplying trophozoite contained in the cyst; typical of a chronic infection with *Toxoplasma gondii*.

Buccal cavity/capsule The space between the oral opening and the beginning of the esophagus in nematodes; useful diagnostically in rhabditiform larvae (*Strongyloides stercoralis*, hookworm).

Buccal teeth/plates Toothlike structure in the mouth cavity of the adult hookworms of the genus *Ancylostoma* (in contrast to buccal plates found in the mouth cavity of the genus *Necator*).

Budding Production of daughter cell as outward growth from the original cell or germinal layer (yeastlike fungi, hydatid cyst).

Buffy coat Grayish white layer of fibrin and leukocytes that forms the upper part of the sedimented cells in whole blood after the addition of anticoagulant (examined in visceral leishmaniasis and trypanosomiasis).

Bursa Umbrella-like expansion of the cuticle at the posterior end of male nematodes; it is supported by muscular rays and aids in copulation (hookworms, trichostrongyles).

Cachexia Severe debilitated state (heavy worm infestation, visceral leishmaniasis).

Calabar swellings Transient swellings of subcutaneous tissues; associated with *Loa loa* infection.

Calcareous corpuscles Rounded masses composed of concentric layers of calcium carbonate that are found in the tissue of tapeworms, characteristic of tapeworm tissue; certain fixatives must be avoided to preserve corpuscles for identification purposes (tapeworms).

Calcifications Portions of host or parasite tissue that become hardened by the deposition of calcium salts; may be visible on radiographic studies (toxoplasmosis, cysticercosis, trichinosis, schistosomiasis).

Capitonnage Closure of a cyst cavity by using sutures (hydatid disease).

Capitulum The blood-sucking, probing, sensing, and holdfast mouthparts of a tick, including the basal supporting structure; the relative size and shape of mouthparts forming the capitulum are characteristic for the genera of hard ticks.

Carcinogen Substance capable of causing a malignant tumor in humans or animals.

Carcinoma Malignant tumor from epithelial tissue (*Clonorchis*, *Opisthorchis*, and *Schistosoma* spp.).

Carditis Inflammation of the heart tissues (Chagas' disease).

Carrier A host that harbors a particular pathogen without showing manifestations of disease.

Casoni antigen Skin test antigen composed of sterile hydatid fluid (hydatid disease).

Cathartic A substance causing evacuation of bowels by increasing bulk, stimulating peristalsis.

Cecum A blindly ending appendage of the intestine.

Cell-mediated immunity Type of immunity that is independent of antibody and is initiated by cytotoxic T cells or by the secretion of lymphokines by T cells. The lymphokines cause other cells, usually lymphocytes and macrophages, to aggregate in the area of T-cell–antigen interaction, leading to a local reaction such as a granuloma.

Cercaria Free-living, tailed larval stage of trematodes; may infect humans by direct penetration (schistosomes); may attach to vegetation and encyst to the metacercarial stage (*Fasciola* and *Fasciolopsis* spp.), or may penetrate tissues of vertebrates or invertebrates and encyst to the metacercarial stage (*Clonorchis* spp.).

Cestode Tapeworm.

Chagas' disease Infection caused by *Trypanosoma cruzi* (American trypanosomiasis).

Chagoma Small granuloma in the skin, caused by early multiplication of *Trypanosoma cruzi* (Chagas' disease).

Charcot-Leyden crystals Slender crystals that are formed from the breakdown products of eosinophils; shaped like double, elongated pyramids with pointed ends; can be found in feces, sputum, and tissues; indicates an immune response that may or may not be related to a parasitic infection.

Chiclero ulcer Single, self-limiting cutaneous papule, nodule, or ulcer (usually on the face or ears) (leishmaniasis).

Chigger The six-legged larva of *Trombicula* spp.; a blood-sucking stage of mites that includes the vectors of scrub typhus.

Cholangiocarcinoma Cancer of the bile duct, which can be the result of chronic infections with *Opisthorchis viverrini*, the Southeast Asian liver fluke.

Cholecystitis Inflammation of the gallbladder.

Chorea Irregular, spasmodic, involuntary movements of the limbs or facial muscles.

Chorioretinitis Inflammation of the posterior coat and retina of the eye (toxoplasmosis).

Chromatin Deep-staining DNA-containing portion of the nucleus (protozoa).

Chromatoidal bar/body Deep-staining, bar-shaped, round, or splinter-shaped inclusions found in the cytoplasm of certain amebae (*Entamoeba* spp.).

Chyluria The presence of lymph and emulsified fat in the urine (filariasis).

Cilia Small, hairlike cytoplasmic projections from a cell or organism; used for motility (*Balantidium coli*).

Cirrhosis Disruption of the normal structure of the liver; destruction of liver cells and increase in connective tissue (schistosomiasis).

CLBs These organisms were thought to be a new pathogen, possibly an oocyst, a flagellate, an unsporulated coccidian, a large *Cryptosporidium* sp., a blue-green alga (cyanobacterium-like body), or a coccidian-like body. They are now known to be coccidia in the genus *Cyclospora*.

Coccidian A group of protozoan parasites that infect the epithelial cells of the intestinal tract (and other tissues) (*Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora belli*).

Coenurus Tapeworm larva of the genus *Multiceps*, characterized by multiple scolices invaginated into the fluid-filled bladder; no daughter cysts produced.

Colitis Inflammation of the colon.

Colon Portion of the large intestine from the cecum to the rectum.

Coma Absolute unconsciousness.

Commensalism Association in which one individual receives benefits and the other is neither helped nor harmed.

Complement A series of proteins that bind in a complex series of reactions to antibody (immunoglobulin G or M) when the antibody is itself bound to an antigen; produces cell lysis if the antibody is bound to antigens on the cell surface.

Concentration techniques Procedures, usually in fecal examinations, allowing the examination of large amounts of feces (flotation or sedimentation procedures; some available for blood specimens and urine specimens).

Concomitant immunity Type of immunity where the host harbors a population of adult worms but is immune to reinfection.

Congenital Present at, and usually before, birth (regardless of cause).

Conjunctivitis Inflammation of the membrane that lines the eyelids and covers the surface of the eyeball.

Convulsion A violent, involuntary contraction of an extensive group of muscles; disturbance of cerebral function (spider and scorpion envenomation).

Coracidium A ciliated larval tapeworm stage that occurs in the eggs of cestodes such as *Diphyllobothrium* and *Spirometra* spp. (pseudophyllidian tapeworms); this is a free-swimming stage, containing six hooks like those found in the oncospheres of other tapeworms.

Cor pulmonale Cardiopulmonary problems which may terminate in congestive heart failure; obstructive vascular disease (*Schistosoma mansoni* infection).

Corrosive Any substance that causes visible destruction of human tissue at the site of contact. The U.S. Environmental Protection Agency defines corrosivity as a substance that is highly acidic (pH < 2.1) or highly alkaline (pH > 12.4).

Creeping eruption Penetration and migration through subcutaneous tissues by skin-penetrating nematodes, resulting in intense pruritus and sometimes secondary bacterial or fungal infection (*Strongyloides* and hookworm infections, cutaneous larva migrans).

Crithidia Old term for epimastigote.

Cryptosporidiosis Intestinal infection caused by coccidia (*Cryptosporidium* spp.).

Cutaneous Pertaining to the skin.

Cuticle Outermost, three-layered portion of the body wall of nematodes (*Ascaris lumbricoides*, etc.).

Cysticercoid The solid larval stage of tapeworms containing an invaginated scolex that occurs in arthropod intermediate hosts (*Hymenolepis*, *Dipylidium*).

Cysticercosis Tissue infection with larval tapeworms in which the scolex is inverted into a fluid-filled bladder (*Taenia solium*).

Cysticercus Tapeworm larva of the family *Taeniidae* (includes *Taenia solium* and *Taenia saginata*) in which a single scolex is invaginated into a fluid-filled bladder.

Cystoenterostomy Internal drainage of pancreatic cysts into some portion of the intestinal tract (hydatid disease).

Cytoplasm The protoplasm of a cell exclusive of the nuclear material.

Daisy Colloquial term descriptive of the segmented forms (merozoites) of the mature schizont of *Plasmodium malariae*.

Decontamination A procedure that eliminates or reduces microbial or toxic agents to a safe level with respect to the transmission of infection or other adverse affects.

Definitive host Host in which the sexual reproduction of a parasite occurs.

Delayed hypersensitivity A manifestation of cell-mediated immunity, different from immediate hypersensitivity in that the maximal response is reached about 24 h or more after intradermal injection of the antigen; infection site is infiltrated by monocytes and macrophages.

Dermatitis Inflammation of the skin (filariasis, schistosomiasis, infections with *Strongyloides* and hookworm larvae, leishmaniasis, *Sarcoptes* infections).

Diarrhea Frequent passage of soft or liquid stool (no blood); may be caused by any parasite or infection normally found in any part of the intestine (*Giardia lamblia*, *Isospora belli*, *Dientamoeba fragilis*, *Balantidium coli*, *Cryptosporidium* spp., *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, possibly other microsporidia, *Cyclospora* spp., *Leishmania* spp. causing visceral leishmaniasis, *Plasmodium falciparum*, tapeworms, *Trichinella* spp., *Schistosoma* spp., hookworm).

Didelphis A genus of marsupials, commonly called opossums, that serve as reservoir hosts of *Trypanosoma cruzi*.

Dioecious Having the male and female sexes of a species as separate individuals.

Direct smear (stool) Approximately 2-mg suspension of fresh, unpreserved feces in water or saline for the purpose of examination for parasites; the primary aim is to see motility.

Disinfectant An agent intended to destroy or irreversibly inactivate all microorganisms, but not necessarily their spores, on inanimate surfaces, e.g., work surfaces or medical devices.

Disseminated infection A parasite enters the body at one point, then disperses throughout the body to other sites.

Diurnal Pertaining to the daylight portion of the 24-h day.

Duodenum The proximal portion of the small intestine (*Strongyloides stercoralis*, *Giardia lamblia*).

Dysentery Frequent watery stools, usually containing blood and/or mucus; associated with inflammation of the intestine, usually the colon (*Entamoeba histolytica*, *Trichuris trichiura*).

Dyspnea Difficulty in breathing.

Dysuria Painful or difficult urination.

Ecosystem The fundamental unit in ecology, comprising the living organisms and the nonliving elements that interact in a defined region.

Ectoparasite Organism that lives on or within the skin of its host (lice, mites, ticks).

Ectopic site Outside the normal location; i.e., the position of a parasite which lodges in an atypical part of the body.

Ectoplasm Outer layer of the cytoplasm of a cell.

Edema Presence of large amounts of fluid, usually in subcutaneous tissues (filariasis, trypanosomiasis, hookworm).

Elephantiasis Inflammation and obstruction of the lymphatic system, resulting in hypertrophy and thickening of the surrounding tissues, usually involving the extremities and external genitalia (filariasis).

Encapsulation Active process of walling off a parasite by the host through the formation of a connective tissue capsule (trichinosis).

Encephalitis Inflammation of the brain (Chagas' disease, trichinosis).

Encystment Formation of a resistant external wall by protozoa to enable them to survive drying and adverse environmental conditions; encysted forms are infective to humans.

Endemic Disease present in a localized community or area at all times.

Endoparasite Parasite that lives within the body of the host.

Endoplasm Inner portion of the cytoplasm of a cell.

Endospore The chitinous inner spore coat (microsporidia).

Engineering controls Controls (e.g., sharps disposal containers, self-sheathing needles, safer medical devices) that isolate or remove a hazard from the workplace.

Eosinophilia Formation of large numbers of eosinophilic leukocytes as a result of some type of immune response; usually found in helminth infections, particularly with tissue invasion (visceral larva migrans, trichinosis, filariasis, schistosomiasis, ascariasis, strongyloidiasis).

Eosinophilic cerebrospinal fluid pleocytosis Increased number of eosinophils in cerebrospinal fluid (*Angiostrongylus* and *Baylisascaris* infections; coenurosis, cysticercosis, echinococcosis, *Fasciola*, *Gnathostoma*, *Paragonimus*, *Schistosoma*, *Toxocara*, *Toxoplasma*, and *Trichinella* infections; possibly *Dirofilaria*, *Onchocerca*, and *Ascaris* infections).

Eosinophilic meningitis Inflammation of membranes of the brain or spinal cord accompanied by an increased number of eosinophils, usually associated with particular helminth infections (angiostrongyliasis, gnathostomiasis, cysticercosis, schistosomiasis).

Epidemic Disease that spreads rapidly and infects many people in a community or area (usually within a short time frame).

Epidemiology The scientific and medical study of the causes and transmission of disease within a population.

Epidermal Pertaining to the outer layer of the skin.

Epididymitis Inflammation of the epididymis (first part of the excretory duct of each testis) (Bancroftian filariasis).

Epimastigote Developmental stage of the family *Trypanosomatidae*; the base of the flagellum is in front of the nucleus, and as the flagellum passes through the body to emerge as the free flagellar portion, it is attached to the body by the undulating membrane. (Old term: crithidia.)

Erythema Diffuse or patchy redness of the skin; blanching on pressure as a result of congestion of cutaneous capillaries.

Erythrocytic cycle Developmental cycle of malarial parasites within the red blood cells.

Eukaryote A cell containing a membrane-bound nucleus with chromosomes of DNA, RNA, and proteins, with cell division involving a form of mitosis in which mitotic spindles (or some microtubule arrangement) are involved; mitochondria are present.

Evagination Protrusion of some part or organ from its normal position (tapeworm scolex in larval worms).

Exflagellation The extrusion of rapidly waving flagellum-like microgametes from microgametocytes; in human malaria parasites, this occurs in the blood meal taken by the proper anopheline vector within a few minutes after ingestion of the infected blood by the mosquito.

Exoerythrocytic cycle A part of the malarial life cycle in which the mosquito introduces sporozoites into the vertebrate host; sporozoites penetrate the parenchymal liver cells and undergo schizogony, resulting in the production of liver merozoites, which then initiate the erythrocytic cycle.

Exospore The proteinaceous outer spore coat (microsporidia).

Facultative parasite One that can complete its life cycle in a free-living environment or, alternatively, that lives during part (or all) of its cycle within a host.

Feces The unabsorbed residue of the digestive process, along with sloughed epithelium, mucus, and bacteria.

Festoon A distinguishing characteristic of certain hard tick species, consisting of small rectangular areas separated by grooves along the posterior margin of the dorsum of both males and females.

Fever A complex physiologic response to disease mediated by pyrogenic cytokines and characterized by a rise in core temperature, generation of acute-phase reactants, and activation of immunologic systems.

Filariform larvae Slender, infective larvae of *Strongyloides stercoralis* and hookworm.

Flame cell Primitive, ciliated excretory cell in trematodes; the movement of the cilia on this cell within the miracidium larva (within a schistosome egg) indicates egg viability.

Flesh flies Members of the order *Diptera*, whose larvae (maggots) develop in putrefying or living tissues.

Fluke Trematode (*Clonorchis*, *Paragonimus*, *Fasciola*, and *Schistosoma* spp.).

Fundus, ocular The portion of the interior of the eyeball around the posterior pole, visible through the ophthalmoscope.

Funiculitis Inflammation of the spermatic cord (filariasis).

Furcocercous Fork-tailed (cercaria of schistosomes).

Gametocyte In malaria, the sexual cell (male microgametocyte or female macrogametocyte). It is present in peripheral blood; fertilization occurs in the mosquito stomach with formation of the zygote.

Gastroenteritis Inflammation of the stomach and small and large intestines.

Genital primordium Ovoid clump of cells that becomes the reproductive system (seen in the rhabditiform larvae of *Strongyloides stercoralis*).

Geohelminth Any helminth that is transmitted to humans from the soil (*Ascaris*, *Strongyloides*, *Trichuris*, hookworm).

Germicide A general term for an agent that kills pathogenic microorganisms on inanimate surfaces.

Granulomatous amebic encephalitis (GAE) *Acanthamoeba* sp. GAE tends to affect immunosuppressed, chronically ill, or otherwise debilitated persons, generally with no particular history of exposure to fresh water sources. Whether caused by an *Acanthamoeba* sp. or *B. mandrillaris*, GAE is a slowly progressive disease of the central nervous system.

Granuloma Tumorlike nodule of firm tissue formed as a reaction to chronic inflammation, usually of lymphoid and epithelioid cells.

Gravid Filled with eggs, such as a gravid pinworm or gravid proglottid of a tapeworm.

Gynecophoral groove or canal The ventral incurved fold of the body extending from the ventral sucker to the caudal extremity, for carrying the female worm (schistosomes).

Halzoun Presence of worms on pharyngeal mucosa; congestion of tissues accompanied by difficulty in breathing and possible asphyxiation (*Fasciola hepatica* and pentastome infection).

Harada-Mori culture Method of incubating fecal material on a filter paper strip in a test tube containing water (cover one-third of the length of paper strip) for the purpose of culturing and recovering nematode larvae (*Strongyloides stercoralis*, hookworm).

Helminth May refer to a nematode (roundworm), cestode (tapeworm), or trematode (fluke).

Hematuria Passage of free hemoglobin in the urine (*Plasmodium falciparum* infection, blackwater fever).

Hemoflagellate Any flagellated protozoan blood parasite (*Trypanosoma* spp.).

Hepatitis Inflammation of the liver (amebiasis, schistosomiasis, infections with liver flukes).

Hepatomegaly Enlargement of the liver.

Hermaphroditism Presence of both male and female reproductive systems in the same individual; most trematodes and cestodes are hermaphroditic.

Heterakis A genus of important nematode parasites. *Heterakis gallinarum* is the cecal worm of chickens and turkeys and is the vector of *Histomonas meleagridis*, a protozoan that causes histomoniasis (a model of the possible transmission of *Dientamoeba fragilis* within helminth eggs such as *Enterobius vermicularis* and *Ascaris lumbricoides*).

Heterophyids Small intestinal flukes parasitic in humans and animals (*Heterophyes* and *Metagonimus* spp.).

Hexacanth embryo Six-hooked tapeworm embryo (oncosphere).

HIV Human immunodeficiency virus.

Hives *See* Urticaria.

HMO Health maintenance organization.

Host An organism in or on which a parasite lives.

Hydatid, alveolar Type of hydatid cyst formed by *Echinococcus multilocularis*; budding is external, as well as internal, and there is no thick outer capsule.

Hydatid, polycystic Type of hydatid cyst formed by *Echinococcus oligarthrus* and *Echinococcus vogeli*; the structure is between those of unilocular and alveolar types of hydatid cyst.

Hydatid, unilocular Type of hydatid cyst formed by *Echinococcus granulosus*; single limiting membrane.

Hydatid cyst Tapeworm larval stage of the genus *Echinococcus*; consists of a large bladder with an inner germinal layer from which daughter cysts and scolices develop (some of which break off and drop into the fluid-filled bladder).

Hydatid sand Consists of scolices, daughter cysts, hooks, and calcareous corpuscles found in the fluid within the hydatid cyst (*Echinococcus granulosus*).

Hydatid thrill A delicate vibration felt by the hand after quick palpation or percussion over the area of the body where the hydatid cyst is located (*Echinococcus granulosus*).

Hydrocele Accumulation of serous fluid in a saclike cavity, particularly the scrotal sac (filariasis).

Hydrocephalus An abnormal accumulation of fluid in the cerebral ventricles or in the subarachnoid space of the brain (cysticercosis).

Hyperparasitemia Infection with *Plasmodium falciparum* at a density of more than 250,000 parasites/μl.

Hyperpigmentation Intensification of pigment; most obvious in dark-skinned races (leishmaniasis, onchocerciasis).

Hypersensitivity Enhanced state of responsiveness following sensitization to a particular antigen. There are four types: type I (anaphylaxis), type II (antibody-dependent cytotoxicity), type III (immune complex hypersensitivity), and type IV (delayed hypersensitivity).

Hypnozoite Exoerythrocytic schizozoite of *Plasmodium vivax* or *Plasmodium ovale* in the human liver, characterized by delayed primary development; responsible for malarial true relapse.

Hypopyon The presence of pus in the anterior chamber of the eye.

Hypostome The central unpaired holdfast organ of the tick capitulum; covered with recurved spines that serve as an anchoring device while the tick feeds.

IATA The International Air Transport Association, a body of the commercial airline industry that governs international aviation. It publishes the *Technical Instructions for the Safe Transport of Dangerous Goods by Air* and regulates dangerous goods for member airlines and anyone who tenders dangerous goods to those airlines.

Immunity The ability of an individual to resist and/or control the effects of antigens (antigen sources could be animal, plant, or mineral).

Immunoglobulin A (IgA) A class of immunoglobulin that is the second most common immunoglobulin in blood. It is the main immunoglobulin found in secretions, such as tears, saliva, colostrum, mucous membranes of the intestine, respiratory and reproductive tracts. IgA provides local defense against microorganisms as they try to infect mucous membranes.

Immunoglobulin E (IgE) A class of immunoglobulin that is concentrated in the lungs, skin, and mucous membranes. It provides the primary defense against environmental antigens and reacts with certain antigens to trigger the release of chemical mediators that cause anaphylactic hypersensitivity reactions characterized by wheal and flare; may indicate a parasitic infection.

Immunoglobulin G (IgG) This is the most abundant immunoglobulin and is approximately equally distributed in blood and in tissue liquids. Approximately 75% of serum immunoglobulins in humans is IgG.

Immunoglobulin M (IgM) This is the third most common immunoglobulin in serum. IgM is the first class of immunoglobulin that is produced by immune cells when stimulated by antigen.

Immunologically naive An individual who has not been exposed to a particular parasitic antigen; often used to refer to patients who may have malaria for the first time and have no residual antibody (*Plasmodium* spp. in travelers).

Incubation period Time span from introduction of disease-causing organisms until symptoms of the disease occur.

Induced malaria Malaria infection acquired by blood transfusions or possible sharing of needles by drug addicts (*Plasmodium* spp.).

Infectious waste Waste containing or assumed to contain pathogens of sufficient virulence and quantity that exposure to the waste by a susceptible host may result in a communicable disease.

Infestation Presence of arthropods on the surface of the body; does not refer to endoparasites (*Pediculus* spp.).

Inflammation Result of tissue reaction to injury; symptoms are redness, pain, swelling, and fever.

Innate immunity Inborn resistance mechanisms that are encountered by a pathogen, before adaptive immunity is induced.

Inspissate To thicken or dry a substance by removing liquids by evaporation (preparation of certain types of media used to grow some amebae).

Intermediate host A required host in the life cycle in which larval development takes place; this must occur before the stage is infective for the definitive host or secondary intermediate hosts.

Invagination The ensheathing, enfolding, or insertion of a structure within itself or another (tapeworm larval worms).

Jaundice A condition caused by excess bilirubin and bile pigment deposition in the skin, which may give the patient a yellow appearance, especially the eyes (*Plasmodium falciparum*).

The Joint Commission Formerly the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). An independent, not-for-profit organization, The Joint Commission accredits and certifies more than 20,500 health care organizations and programs in the United States. Joint Commission accreditation and certification are recognized nationwide as a symbol of quality that reflects an organization's commitment to meeting certain performance standards.

Kala azar Another name for visceral leishmaniasis (*Leishmania donovani*).

Karyosome Concentrated clumps of chromatin material within the nucleus; position and morphology are often used to differentiate intestinal protozoa.

Kerandel's sign Delayed sensation to pain (African trypanosomiasis).

Keratitis Inflammation of the cornea (Chagas' disease, onchocerciasis, *Acanthamoeba* infection, microsporidiosis).

Keratoconjunctivitis Inflammation of the conjunctiva and of the cornea.

Kinetoplast Intensely staining rod- or disk-shaped or spherical extranuclear DNA structure found in parasitic flagellates near the base of the flagellum.

Knott technique Concentration procedure using blood and dilute formalin; designed to detect microfilaria (*Wuchereria* and *Brugia* spp.).

Kupffer cells Phagocytic epithelial cells lining the sinusoids of the liver.

Lagochilascaris minor An unusual nematode parasite of humans (ingestion of infective eggs from the environment or ingestion of infective larvae in the tissues of an intermediate host). In most cases, the worms are located in the soft tissues of the neck and throat, tonsils, mastoids, and paranasal sinuses (chronic or recurrent abscesses). Fatal encephalopathy (one case) has also been reported. All cases were in the tropical regions of the Western Hemisphere.

Larva currens Cutaneous larva migrans caused by rapidly moving larvae of *Strongyloides stercoralis*, typically extending from the anal area down the upper thighs; may also be caused by zoonotic species of *Strongyloides*.

Larva migrans, cutaneous/visceral/ocular/neural Disease characterized by thin, red, convoluted papular or vesicular lines of eruption that extend at one end while fading at the other (dog or cat hookworm). Visceral larva migrans involves migration of larvae through the deep tissues, including the eye (dog or cat ascarids, *Toxocara* spp.). The central nervous system may also be involved.

Latex allergy Allergic reaction associated with latex glove use. The two types of allergic reactions are contact dermatitis (type IV delayed hypersensitivity) due to chemicals used in processing latex and the more serious IgE/histamine-mediated allergy (immediate or type I hypersensitivity) due to latex proteins.

Leishman-Donovan body Small, round, intracellular form of *Leishmania* spp. (reticuloendothelial system) and *Trypanosoma cruzi* (striated muscle); also called amastigotes or L-D bodies.

Leptomonad Old term for the promastigote stage, which is long and slender and found in the insect vector for *Leishmania* spp.; it is also recovered in artificial culture media (Novy-MacNeal-Nicolle [NNN] medium).

Leukocytosis Increase in the number of white blood cells, usually to more than 10,000/mm^3.

Leukopenia Decrease in the number of white blood cells, usually to less than 4,000/mm^3.

Lichen simplex A discrete flat papule or an aggregate of papules giving a patterned configuration resembling lichens growing on rocks.

Loeffler's syndrome Transient pulmonary infiltration; day-to-day clearing in 3 to 14 days; associated with marked peripheral eosinophilia (*Ascaris lumbricoides*) (also Loffler's, Löffler's).

Lumen Cavity of hollow, tubular organs, such as the intestine or blood vessels.

Lymph Plasma and white blood cells that bathe the tissue cells.

Lymphadenitis Inflammation of the lymph nodes (filariasis).

Lymphangitis Inflammation of the lymphatic vessels (filariasis).

Lymphedema Swelling from excess fluid due to malfunction of the lymphatic system.

Lymphocytosis Absolute or relative increase of lymphocytes in the blood.

Lymphokine One of several kinds of effector molecules released by T lymphocytes when an antigen to which the lymphocytes are sensitized binds to the cell surface.

Lymph varices Dilated lymph vessels secondary to lymphatic blockage (filariasis).

Macrogametocyte The mother cell producing the macrogametes, or female elements of sexual reproduction in the sporozoan and microsporidian protozoa; macrogamont.

Macronucleus Large, kidney bean–shaped nucleus in *Balantidium coli* (the shape is not always consistent).

Macrophage Motile, phagocytic, mononuclear cell that originates in the tissues and may be confused morphologically with protozoan trophozoites (particularly *Entamoeba* spp.).

Malabsorption Poor fat absorption in the upper small bowel (giardiasis).

Malaise Vague feeling of discomfort throughout the whole body; tiredness.

Malaria Benign tertian malaria, *Plasmodium vivax*; malignant tertian malaria, *Plasmodium falciparum*; ovale malaria, *Plasmodium ovale*; quartan malaria, *Plasmodium malariae*; simian malaria, *Plasmodium knowlesi*; relapsing malaria, renewal of clinical activity at some interval after the primary attack; remittent malaria, a malarial fever (usually due to *P. falciparum*) in which the temperature falls but not to the normal level during the interval between two pronounced paroxysms.

Malarial pigment Composed of hematin and excess protein left over from the metabolism of hemoglobin; appears as brownish pigment after Giemsa staining (*Plasmodium* spp.).

Malignant tertian malaria Malaria caused by *Plasmodium falciparum*.

Mast cell A connective tissue cell that contains coarse, basophilic, metachromatic granules; the cell is thought to contain heparin and histamine.

Mastocytosis Abnormal proliferation of mast cells in a variety of tissues; may be systemic, involving a variety of organs, or cutaneous (urticaria pigmentosa).

Maurer's dots or clefts Irregular dots that occur infrequently in red blood cells infected with *Plasmodium falciparum* (dots tend to be more blue after Giemsa staining than do Schüffner's dots).

Measly Pertaining to pork or beef infected with the cysticerci of the tapeworms *Taenia solium* or *Taenia saginata*.

Megacolon Dilation of the colon (Chagas' disease).

Megaesophagus Dilation of the esophagus (Chagas' disease).

Meningitis Inflammation of the membranes of the brain or spinal cord.

Meningoencephalitis Inflammation of the brain and its surrounding membranes (trypanosomiasis, malaria, *Naegleria* and *Angiostrongylus cantonensis* infections).

Merogony Synonym for schizogony, leading to the production of daughter cells (merozoites).

Meront Diplokaryotic cells that grow and divide into daughter cells (merozoites).

Merozoite Product of schizogonic cycle in malaria; produced in the liver (pre-erythrocytic cycle) and in the red blood cells (erythrocytic cycle).

Mesentery Tissue that supports the intestinal tract.

Metacercaria The infective, encysted larval form of a trematode; found within the tissues of an intermediate host or on plant material (*Paragonimus*, *Clonorchis*, and *Fasciolopsis* spp.).

Methenamine silver stain Both hot and cold methods; involves deposition of silver onto cyst wall (*Pneumocystis jiroveci* [now classified with the fungi]).

Microfilaria Embryos produced by filarial worms (nematodes); usually found in the blood or tissues of patients with filariasis (*Wuchereria*, *Brugia*, and *Onchocerca* spp.).

Microgametocyte The mother cell producing the microgametes, or male elements of sexual reproduction in the sporozoan and microsporidian protozoa; microgamont.

Micrometer (micron) Unit of measure equal to 0.001 mm; abbreviated µm.

Micron *See* Micrometer.

Micronucleus Small, dotlike nucleus found in *Balantidium coli*; often very difficult to see, even in stained preparations.

Miracidium Free-living, ciliated larva released from a trematode egg and infective for the snail intermediate host.

Monocytosis Increase in the number of monocytes in the peripheral blood; may be found in both helminth and protozoan infections.

Monoecious Both male and female reproductive organs occur in the same individual (hermaphroditic) (trematodes, cestodes).

Montenegro test Delayed-hypersensitivity skin test; injection of leishmanial antigen; read after 72 h; positive reaction in cured individuals, negative in early cases (visceral and mucocutaneous leishmaniasis).

Mordant A substance capable of combining with a dye and the material to be dyed, thereby increasing the affinity or binding of the dye.

MSDS Material Safety Data Sheet Provides detailed information about hazards and protective measures relative to a hazardous chemical substance.

Multilocular cyst Cyst containing many cavities (*Echinococcus multilocularis*).

Mutualism Association in which both parties benefit and cannot survive without each other.

Myiasis Infestation with maggots (fly larvae).

Myocarditis Inflammation of the heart muscles (trichinosis, trypanosomiasis, toxoplasmosis).

Myositis Inflammation of voluntary muscles (trichinosis, *Sarcocystis* infections).

Nanophyetus salmincola Digenetic trematode that causes infections in humans (ingestion of raw, undercooked, or smoked salmon); infections have been reported from North America. Symptoms include gastrointestinal complaints, unexplained eosinophilia, and recovery of eggs from stool specimens.

Necropsy *See* Autopsy.

Negative predictive value (NPV) (Example) The probability that the individual is not infected by *Plasmodium* when the rapid test is negative.

Nit The ovum or hatched egg of a body, head, or crab louse; it is attached to human hair or clothing by a layer of chitin.

Nocturnal Pertaining to the dark portion of a 24-h day; active at night (filariasis).

Nodule A small, hard node that can be detected by touch (onchocerciasis, coenurosis, cysticercosis, myiasis).

Nucleophaga A microsporan parasite of amebae that destroys the nucleus of its host.

Nucleus A cellular inclusion composed of chromatin; the morphology is often used to help identify intestinal protozoa (*Entamoeba* and *Dientamoeba* spp.).

Obligate parasite Parasite that must always live in contact with the host.

Occult blood Blood present in very small amounts; usually detectable only by chemical means. The specimen is usually a stool sample. The finding may or may not be related to parasitic infection.

Occupational exposure Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with a hazard that may result from the performance of an employee's duties.

Omentoplasty Use of the greater omentum to cover or fill a defect (hydatid disease).

Onchocercoma Nodule containing adult worms (onchocerciasis).

Oncosphere Spherical, six-hooked tapeworm larva within the eggshell (*Taenia* and *Hymenolepis* spp.).

Oocyst A thick- or thin-walled stage in the life cycle of the coccidia. Stage shed in feces in human infections.

Ookinete The motile zygotes of the malarial parasite that penetrate the mosquito stomach to form an oocyst under the outer gut lining. In an opened tube of human blood, this stage can develop over time as the blood cools and is oxygenated (the malarial parasites "think" they are in the mosquito, and exflagellation and development of the ookinete can occur within the tube of blood; this is not common but is well documented, and the morphology can mimic the *Plasmodium falciparum* gametocyte).

Operculate egg Egg with a lid (trapdoor) at one end through which the larva escapes (*Diphyllobothrium*, *Clonorchis*, and *Paragonimus* spp.).

Operculum A lidlike structure at one end of the egg shell through which the larval form escapes (*Diphyllobothrium*, *Clonorchis*, and *Paragonimus* spp.).

OPIM *See* Other potentially infectious material.

Opportunistic Denoting an organism capable of causing disease only in a host whose resistance is lowered (by other diseases or drugs, etc.); also denotes the disease.

Opsonization Modification of the surface characteristics of an invading particle or organism by binding with antibody or a nonspecific molecule to facilitate phagocytosis by host cells.

Orchitis Inflammation of a testis; may be accompanied by swelling, pain, and fever (filariasis).

OSHA Occupational Safety and Health Administration.

Other potentially infectious material (OPIM) Human body fluids including semen, vaginal secretions, urine, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva, body fluids which may be contaminated with blood, unfixed tissue, HIV- or hepatitis virus–containing cell or organ cultures, blood and tissue from an infected animal, reagents, infectious waste, and cultures.

Oviparous Egg-laying.

Pancytopenia Pronounced reduction in the number of erythrocytes, all types of white blood cells and blood platelets in the circulating blood.

Pan-malarial antigen An antigen expressed by all five of the *Plasmodium* spp. which are pathogenic in humans, i.e., *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*.

Papule Small, solid elevation of the skin.

Parasite An organism living on or in, and at the expense of, another organism.

Parasitemia The presence of parasites in the circulating blood (malaria, trypanosomes, microfilariae).

Parasitophorous vacuole Extracellular but intracytoplasmic vacuole, surrounded by host cell membrane (*Cryptosporidium*).

Paratenic host A host in which a parasite survives without undergoing any additional development (a transport host only) (e.g., *Lagochilascaris minor*).

Parenteral Piercing mucous membranes or the skin through such events as needlesticks, human bites, and abrasions.

Paresis Slight paralysis (cysticercosis).

Paroxysm Rapid onset or return of symptoms or increased intensity of symptoms; usually applies to the periodicity of malaria symptoms (chills, fever, sweats).

Parthenogenesis Form of sexual reproduction in which the organism develops without fertilization by the male gamete (*Strongyloides stercoralis,* parasitic generation).

Patency The development stage at which helminths begin egg production; time to maturity (intestinal helminths).

Pathogen Any organism or substance that produces a disease state.

Pemphigus Autoimmune bullous diseases; nonspecific term for blistering skin diseases.

Pentatrichomonas A genus of parasitic protozoan flagellates, formerly part of the genus *Trichomonas*. New generic name for *Trichomonas hominis,* a nonpathogenic flagellate living in the human colon.

Pericardial tamponade Condition resulting from accumulation of excess fluid in the pericardium.

Periodic acid-Schiff stain (PAS) Involves an oxidation process by periodic acid followed by Schiff reagent (detection of newly formed aldehyde groups) and sulfurous rinses; glycogen, chitin, and mucoproteins are strongly PAS positive; lipids are PAS positive (amebae, worm cuticle, part of microsporidial spore, fungi).

Periodicity Recurrence of an event at regular intervals (nocturnal periodicity of microfilariae of *Wuchereria bancrofti;* malarial symptoms).

Peristalsis Movement of food through the intestine by contractions of the intestinal musculature.

Peritonitis Inflammation of the membrane that lines the abdominal cavity and covers the viscera.

Permissible exposure limit (PEL) Maximum allowed exposure during a time-weighted average (8-h workday or 40-h workweek).

Personal protective equipment (PPE) Specialized clothing or equipment worn by an employee for protection against a hazard.

Petechiae Minute, hemorrhagic spots in the skin or mucosa.

Pica A craving for substances not fit for food, such as starch, clay, ashes, or plaster; ingestion of clay or soil can be associated with exposure to *Ascaris*, *Trichuris*, hookworm, *Strongyloides*, *Toxocara*, and *Toxoplasma* organisms.

Pinworm Human roundworm parasite of the colon and rectum, extremely common in children; diagnostic approach is with a Scotch tape preparation (*Enterobius vermicularis*).

Pipe-stem fibrosis Granulomatous reaction around schistosome eggs in periportal tissues; fibrous tissue is white and hard (*Schistosoma japonicum*, *Schistosoma mansoni*).

Plasma Liquid portion of the blood or lymph.

Plerocercoid Tapeworm larva of the genera *Diphyllobothrium* and *Spirometra*; also known as a sparganum larva.

Pneumonitis Inflammation of the pulmonary tissue (*Ascaris*, *Strongyloides*, *Pneumocystis* spp.).

Polar filament (microsporidia) A thin tubule within the spore, attached anteriorly, extending back and lying coiled within the spore; part of the extrusion apparatus that everts and inoculates the sporoplasm into a host cell (microsporidia); same as polar tubule.

Polar filaments (*Hymenolepis nana*) Filaments arising from opposite poles of the oncosphere membrane of *H. nana;* they lie between the egg shell and the oncosphere.

Polar plugs Mucoid plugs present at both ends of the egg (*Trichuris trichiura*).

Polymerase chain reaction (PCR) a technique used to make numerous copies of a specific segment of DNA (or RNA) quickly and accurately.

Positive predictive value (PPV) (Example) The probability that an individual actually is infected by *Plasmodium* when the rapid test is positive.

Prepatent period Time between infection of the host and the beginning of egg production (intestinal helminths).

Primary amebic meningoencephalitis (PAM) An invasive, rapidly fatal cerebral infection by soil and water amebae, chiefly *Naegleria fowleri*, found in humans and other primates. The disease is characterized by high fever, neck rigidity, and symptoms associated with upper respiratory infection (cough and nausea). The brain is the primary focus, especially the olfactory lobes and cerebral cortex; organisms enter through the nasal mucosa and through the cribriform plate. Death usually occurs 2 to 3 days after onset of symptoms.

Primary container A vessel, including its closure, which contains the specimen.

Prions Infectious, abnormal host proteins that cause transmissible spongiform encephalopathies and are resistant to a number of standard disinfection and sterilization procedures.

Proglottid Tapeworm segments containing male and female reproductive systems; may be immature, mature, or gravid (*Taenia* and *Hymenolepis* spp.).

Promastigote Development stage of the family *Trypanosomatidae*; the base of the flagellum is anterior to the nucleus, the free flagellar portion is short, and there is no undulating membrane.

Prophylaxis The use of drugs in healthy individuals to prevent initial infection (*Plasmodium* spp).

Proteinuria Protein in the urine (*Plasmodium falciparum*).

Protozoa Single-celled eukaryotes (organisms whose cells have nuclei) that that are primarily unicellular, existing singly or aggregating into colonies, are usually nonphotosynthetic, and are often classified further into phyla according to their capacity for and means of motility, as by pseudopods, flagella, or cilia.

Prurigo nodularis An eruption of hard nodules in the skin, caused by rubbing and accompanied by intense itching.

Pruritus Severe itching (cutaneous larva migrans, infection with scabies, itch mite, or pinworm).

Pseudocyst Usually refers to a large number of *Toxoplasma gondii* trophozoites enclosed in a macrophage or some other host cell; parasites bound by host cell tissue not of parasite origin.

Pyrogenic Producing fever.

Pyuria Presence of pus (bacteria, white blood cells) in the urine.

Quartan malaria Malaria caused by *Plasmodium malariae*.

Quotidian malaria Type of malaria in which fevers occur every 24 h; may indicate early infection or mixed infection.

Rash A superficial skin eruption; may be macular, maculopapular, blotchy, etc.

Rectal prolapse Extrusion of the rectal mucosa through the anus; may occur in children with heavy *Trichuris trichiura* infections. Usually no sequelae after therapy.

Rediae An elongated sac with a mouth, muscular pharynx, primitive gut, birth pore, and germinal cells, which may develop into cercariae or daughter rediae (stage within snail host: trematodes).

Regulated waste Liquid or semiliquid blood or OPIMs; contaminated items that would release blood or OPIMs in a liquid or semiliquid state if compressed; items that are caked with dried blood or OPIMs and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or OPIMs.

Reservoir host The host of an infection in which the infectious agent multiplies and/or develops and on which the agent is dependent for survival in nature; essential host for maintenance of the infection when active transmission is not occurring.

Retinochoroiditis Inflammation of both the retina and the choroid/vascular coat of the eye (toxoplasmosis, onchocerciasis, visceral larva migrans).

Rhabditoid (rhabditiform) larvae Noninfective, thick, rod-shaped larvae of hookworm and *Strongyloides stercoralis*; rhabditoid refers to the shape of the larval esophagus.

Romaña's sign Marked edema of one or both eyes; usually dry and unilateral (Chagas' disease).

Rosette Arrangement of merozoites in mature schizont of *Plasmodium malariae* in a circle around a clump of excess pigment; also the uterine arrangement in the *Diphyllobothrium latum* proglottid.

Rostellum The anterior fixed or invertible portion of the scolex of a tapeworm, frequently provided with a row (or several rows) of hooklets ("armed rostellum") (*Taenia solium*).

Sappinia diploidea A newly recognized human pathogen causing amebic encephalitis.

Scabies An eruption due to the mite *Sarcoptes scabiei*; the female burrows into the skin, producing a vesicular eruption with intense pruritus between the fingers, on the male genitalia, buttocks, and elsewhere on the trunk and extremities. Crusted scabies is a severe form of scabies with innumerable mites in thickened stratus corneum. This infection has been linked with cellular immune deficiencies, including AIDS.

Schizogony A stage in the asexual cycle of the malarial parasite that occurs within the red blood cells; results in the formation of merozoites (*Plasmodium* spp.).

Schüffner's dots Tiny, red-staining granules in the cytoplasm of red blood cells infected with either *Plasmodium vivax* or *P. ovale*; true stippling (with Giemsa stain).

Scolex The head or attachment portion of a tapeworm; attachment may be by suckers or hooklets (*Taenia*, *Diphyllobothrium*, and *Hymenolepis* spp.).

Scutum In hard ticks, a plate that largely or entirely covers the dorsum of the male and forms an anterior shield behind the capitulum of the female or immature ticks.

Seatworm Another name for pinworm (*Enterobius vermicularis*).

Secondary container A vessel into which the primary container is placed for transport within an institution; it will contain a specimen if the primary container breaks or leaks in transit.

Segmentectomy Excision of a segment of an organ or gland (hydatid disease).

Sensitivity The proportion of true positives that are correctly identified by a test.

Sharps container A container approved for the transport of contaminated sharps.

Sheath Cuticle of a larval nematode that is retained around the body; may serve as protection. Used to help identify microfilariae (*Wuchereria* and *Brugia* spp., *Loa loa*).

Shock Marked decrease in blood pressure; rapid pulse, decreased kidney function (*Plasmodium falciparum* malaria, rupture of hydatid cyst, bee stings).

Short-term exposure limit (STEL) Maximum permitted continuous exposure to a hazardous substance (normally measured in a single 15-min period).

Sigmoidoscopy Visual examination of the rectum and sigmoid flexure of the colon with a lighted tube; often performed for suspected amebiasis cases (*Entamoeba histolytica*).

Simian malaria *Plasmodium knowlesi*.

Sinusitis Inflammation of the sinuses.

Sparganum A migrating tapeworm larva that invades the subcutaneous tissues, causing inflammation and fibrosis (*Diphyllobothrium* and *Spirometra* spp.).

Specificity The proportion of true negatives that are correctly identified by a test.

Spicule Accessory reproductive structure in male nematodes; useful in identification of species.

Spiracle A breathing aperture in arthropods.

Splenomegaly Enlargement of the spleen (leishmaniasis, schistosomiasis, malaria).

Splinter hemorrhages Effects of vasculitis in trichinosis; tiny linear hemorrhages in nail beds (larval migration).

Spore One of the stages in the life cycle of the microsporidia; contains a polar tubule apparatus used for infecting cells; the number of polar tubule coils in the spore is used in organism classification.

Sporocyst An elongated sac without a mouth or other distinct internal or external structure, formed after a miracidium infects a snail. May give rise to cercariae (schistosomes) or rediae (*Paragonimus* spp.).

Sporogony (microsporidia) Production of spores.

Sporogony (*Plasmodium*) A stage in the sexual cycle in the malarial parasite that takes place in the mosquito, with eventual production of the infective sporozoites.

Sporont The zygote stage within the oocyst wall in coccidia; gives rise to sporoblasts, which form sporocysts, within which the infective sporozoites are produced.

Sporozoite Slender, spindle-shaped organism that is the infective stage of malaria parasites; inoculated into humans by the bite of an infected female mosquito; the result of the sexual cycle in the mosquito.

Sporulation The development of spores; a unit of asexual reproduction that may be adapted for dispersal and for survival, often for extended periods of time, in unfavorable conditions.

Spurious parasite Organisms that parasitize other hosts passing through the human intestine and are detected in the stool after ingestion; not a true infection (ingestion of animal liver containing *Capillaria* eggs).

Standard precautions Set of precautions applied to all patients, designed to reduce the risk of transmission of microorganisms in the health care setting.

Steatorrhea Malabsorption characterized by the presence of fat in the stool (giardiasis, strongyloidiasis, cryptosporidiosis, isosporiasis).

Sterilant An agent intended to destroy all microorganisms (viruses, vegetative bacteria, fungi, and large numbers of highly resistant bacterial endospores) on inanimate surfaces.

Sterilization A procedure that effectively kills a microorganism, including bacterial spores on inanimate surfaces.

Stigmal plates Characteristic breathing apparatus in fly larvae in cases of myiasis; used to identify the specific species involved; seen at the skin opening (through which the larva receives oxygen).

Strain An intraspecific group of parasites that differs from other groups in one or more traits, including traits that might be relevant to pathogenicity, treatment, or control.

Strobila The entire chain of tapeworm proglottids, excluding the scolex and neck.

Superinfection Reinfection by a parasite that is already present in the body (*Strongyloides* and *Hymenolepis* spp.).

Swimmer's itch Dermatitis caused by skin penetration of humans by cercariae of schistosomes (normally infects birds and semiaquatic animals).

Symbiosis Close association between two different organisms; living together.

Syncope Fainting or loss of consciousness due to a temporary deficiency of blood supply to the brain (*Dracunculus* infection).

Systemic Involving the entire body.

Tachycardia Rapid heartbeat, usually more than 100 beats/min (trypanosomiasis).

Tachyzoite A rapidly multiplying stage in the development of the tissue phase of certain organisms such as *Toxoplasma gondii.*

Tenesmus Straining to defecate; may be painful and unproductive.

Tetrathyridium Second larval stage in which the scolex with its four suckers is invaginated into the anterior end of a plerocercoid type of body (tapeworm *Mesocestoides*).

Thrill *See* Hydatid thrill.

Tick paralysis A paralytic condition in the host, caused by toxic compounds found in the saliva of certain ticks.

Tinnitus Ringing or buzzing in the ear not resulting from an external source.

Toluidine blue stain Detects the cyst walls of *Pneumocystis jiroveci* as a purple color.

Transmission Transovarial transmission: passage of parasites or infective agents from the maternal body to eggs within ovaries (transmission of viral or rickettsial pathogens to larval mites or ticks).

Traveler's diarrhea Often related to giardiasis or cryptosporidiosis.

Trichinoscope A magnifying glass used in the examination of meat suspected of containing larvae of *Trichinella* spp.

Trichrome stain; Gomori trichrome Developed for histology; Wheatley's modification, developed for staining stool specimens; modified trichrome stains (10× chromotrope dye) used for staining microsporidian spores.

Trophozoite The feeding, motile stage of protozoa.

Trypanosome Slender, flagellate protozoan found in the blood of humans (*Trypanosoma* spp.); also referred to as trypomastigote (new term).

Trypomastigote Newer terminology for the trypanosome stage in human blood (*Trypanosoma* spp.).

Ulcer An open sore on the skin or mucous membrane, characterized by disintegration of the tissue and often by discharge of pus (leishmaniasis, amebiasis, *Dracunculus* infection).

Unilocular Cyst containing only one cavity.

Universal precautions Set of precautions designed to reduce risk of transmission of HIV, hepatitis B virus, and other bloodborne pathogens in the health care setting. Now referred to as standard precautions.

Urethritis Inflammation of the urethra (trichomoniasis).

Urticaria An allergic skin reaction characterized by the development of wheals and intense itching and burning sensations; onset and disappearance are often sudden (filarial infections).

Uta A mild form of New World or American cutaneous leishmaniasis caused by *Leishmania peruviana*, occurring in the high Andean valleys of Peru and Bolivia; small dermal lesions on exposed skin surfaces; the dog is an important reservoir.

Uveitis Inflammation of the uvea (the pigmented, vascular layer of the eye) (*Acanthamoeba* spp.).

Vacuole Cavity in the cytoplasm of a cell that may contain ingested bacteria, yeast cells, or debris; vacuole contents and/or morphology may be helpful in identification of some of the intestinal protozoa (*Entamoeba* spp., *Iodamoeba bütschlii*).

Vaginitis Inflammation of the vagina; prolific, irritating, green or yellowish thin discharge; there may be punctate, hemorrhagic spots (trichomoniasis).

Variant surface glycoprotein (VSG) An antigenic coat protein found on the surface of certain African trypanosomes. Different VSGs are expressed by different generations, explaining antigenic variation seen in these parasites.

Vector A biological vector is a vector such as the *Anopheles* mosquito for malarial agents or the tsetse fly for agents of African sleeping sickness, in which the agent multiplies before being transmitted to another host; a mechanical vector is a vector that conveys pathogens to a susceptible host without essential biological development of the pathogens in the vector (e.g., transfer of organisms on the feet or mouth parts of the housefly).

Vermin Parasitic insects, such as lice and bedbugs.

Vinegar eel Free-living nematode, *Turbatrix aceti*, occasionally occurring as a contaminant in laboratory solutions.

Visceral Pertaining to the internal organs of the body, especially those within the abdominal cavity.

Visceral larva migrans Tissue migration of dog and cat ascarid larvae in humans; the life cycle cannot be completed in the human host; often characterized by high peripheral eosinophilia (*Toxocara cati* and *Toxocara canis*).

Viviparous Discharging living young (*Trichinella*, filariae).

Whipworm Common name for roundworm (*Trichuris trichiura*).

WHO World Health Organization.

Winterbottom's sign Enlargement of the posterior cervical lymph nodes (African trypanosomiasis).

Xenodiagnosis Procedure allowing the feeding of laboratory-raised triatomid bugs (known to be infection free) on the blood of patients suspected of having Chagas' disease; after several weeks, the bug feces are checked for the intermediate stages of *Trypanosoma cruzi;* a type of concentration procedure.

Zooanthroponosis A zoonosis that is normally maintained by humans but can be transmitted to other vertebrates.

Zoonosis A disease of animals that is transmissible to humans (*Cryptosporidium* spp.).

Zygote The cell resulting from the fusion of male and female gametes; formed in the mosquito stomach (*Plasmodium* spp.).

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